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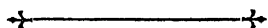
BIOLOGICAL REVIEWS

of the

Cambridge Philosophical Society



Edited
by
H. MUNRO FOX



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DIE HERKUNFT DES ZELLMATERIALS BEI REGENERATIVEN VORGÄNGEN DER WIRBELLOSEN TIERE

VON PROF. H. A. STOLTE.

(Tübingen.)

(Received March 17, 1935.)

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I. EINLEITUNG.

DAS Regenerationsproblem, früher überwiegend morphologisch gesehen, ist seit einer Reihe von Jahren immer mehr von der physiologischen Seite her betrachtet worden, d. h. die mehr äusserliche Beobachtung von Form und Lage des Regenerates und seiner Angleichung an den Gesamtorganismus ist gegenüber der Frage nach den Ursachen dieser Bildungen zurückgetreten. So wird diese Frage erst in neueren Darstellungen des Regenerationsproblems von Korschelt (1927) und Abeloos (1932) ausführlicher behandelt.

Damit ist aber das Regenerationsproblem ganz in die Nähe des Fragenkomplexes gerückt worden, der auch im Mittelpunkt der Erörterungen der entwicklungsphysiologischen Forschung steht, der Frage nach den Ursachen und Kräften für den Aufbau des Organismus.

Von diesem Standpunkt aus werfen Entwicklungsphysiologie und Regenerationsgeschehen die gleichen Fragen auf, aber während die entwicklungsmechanische Forschung immer ein einheitliches embryonales Material zum Ausgangspunkt nimmt, muss die Erforschung der Regeneration eine Vorfrage stellen, nämlich die *nach der Herkunft des Materials*, das der Organismus zum Aufbau des Regenerates benutzt.

Zwei Quellen können für dieses Material angenommen werden: Entweder gewinnt der Organismus Zellmaterial durch Dedifferenzierung der schon funktionierenden Gewebe oder er besitzt Reserven von Zellen, die undifferenziert im Organismus schlummern und bei Bedarf geweckt werden. Den ersteren Fall setzte man früher in Beziehung zur Erscheinung der Metaplasie, in dem letzteren Falle sprach man im Zusammenhange mit dem Weismannschen Hypothesengebäude von Reserveidioplasma. In der folgenden Darstellung sollen zunächst einmal die Tatsachen zusammengestellt werden, ehe nach der einen oder der anderen Auffassung hin eine Entscheidung gefällt wird.

Die Frage nach der Herkunft des Zellmaterials kann nun aber auch, ausser an Regenerationsvorgängen, an den ihnen verwandten Vorgängen der ungeschlechtlichen Fortpflanzung geprüft werden, und schliesslich auch an natürlichen und experimentellen Vorgängen, die einen Neuaufbau des Organismus zur Folge haben. Alle diese Erscheinungen sollen mit in den Kreis der Betrachtung gezogen werden.

Die folgende Darstellung beschränkt sich auf die wirbellosen Tiere, da die entsprechenden Vorgänge bei den Wirbeltieren, weil äusserst kompliziert, noch wenig geklärt sind und der zur Verfügung stehende Raum beschränkt ist.

Im Folgenden werden also zunächst die bei den einzelnen Tiergruppen gewonnenen Tatsachen über die Histogenese bei regenerativen Vorgängen geschildert werden und zuletzt in einem zusammenfassenden Abschnitt die aus diesem Tatsachenmaterial sich ergebenden Schlüsse und Gesetzmässigkeiten besprochen werden.

II. PORIFERA.

Man wird erwarten können, dass bei diesen einfach organisierten Formen die Herkunft des Zellmaterials sehr leicht nachzuweisen ist.

Das Regenerationsvermögen der Schwämme wird allerdings gewöhnlich als geringfügig angesehen (Korschelt (1927), Hentschel (1923-5)). Die Grundlage aller dieser Vorgänge sind nach den meisten Autoren die sog. Archäocyten. Während also von den normalen regenerativen Vorgängen wenig Aufschluss über unsre Frage zu erwarten ist, sind schon frühzeitig zwei andere Erscheinungen dazu benutzt worden, die Histogenese des Schwammkörpers zu studieren, die sog. Reunion nach Dissoziation der Zellen und die Entwicklung des Schwammes aus den Gemmulae.

Von älteren grundlegenden Arbeiten sind hier die von H. V. Wilson (1907) und K. Müller (1911a) zu nennen. Bei dem Durchpressen des Schwammkörpers durch Müllergaze werden eine Anzahl Zellen isoliert und es wurde nun verfolgt, welche dieser isolierten Zellen beim Aufbau des neuen Schwammes wiederverwendet werden. Müller neigt zu der Ansicht, dass die Archäocyten allein die Ausgangszellen des neuen Schwammes sind, eventuell noch die Dermalzellen. Dagegen sollen neue Geisselzellen lediglich durch Archäocyten gebildet werden.

Bei den Untersuchungen Müllers handelte es sich zunächst darum, festzustellen, ob wirklich eine Neubildung des Schwammes von einzelnen Zellen aus

vorlag oder eine Umwandlung des vorhandenen Gewebes, das bei den zunächst unvollkommenen Zerreibungsversuchen (H. V. Wilson) im Zusammenhang blieb. Es konnte also erst die vollständige Trennung der Zellen beim Durchpressen durch Gaze eine Antwort auf diese grundlegende Frage geben.

Ziemlich gleichzeitig sowie zehn Jahre später hat Huxley (1912, 1921a, b) diese Frage untersucht und an einem Kalkschwamm (*Sycon raphanus*) den Aufbau des Organismus aus den einzelnen Zellen verfolgt. Wurden die Zellsorten in normalen Proportionen gemischt, so erfolgte der normale Aufbau. Bei der völligen Dissoziation scheiden sich Dermal- und Gastralzellen. Die ersteren bilden wieder die äussere Hülle des Schwammes, die letzteren die zentrale Masse, die später Hohlräume bildet. Ihre Zellen werden zu Kragengeisselzellen. Kragenzellen allein können keinen Schwamm aufbauen. Wenn auch eine Entdifferenzierung beobachtet wurde (Verlust des Kragens), so ging sie doch nicht so weit, dass diese Zellen totipotent wurden. Danach scheint es, als wäre der Typus der Archäocyten bei diesen Kalkschwämmen nicht so vorherrschend und würde die Aufgabe der isolierten Zellen durch ihre Herkunft mehr bestimmt, als durch ihre Lage.

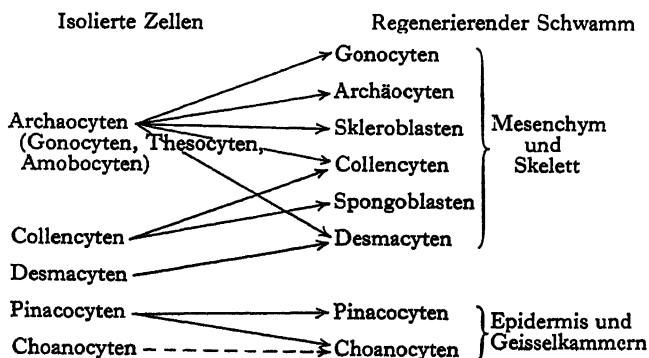
In einer anderen Mitteilung hat Huxley (1921b) festgestellt, dass die Choanocyten die am wenigsten lebenskräftigen unter den isolierten Zellsorten sind.

Galtsoff (1925a) beobachtete zunächst die grosse Beweglichkeit der isolierten Zellen von *Microciona prolifera*, *Reniera* u. a. Schwammformen, die durch Müllergaze Nr. 20 gepresst waren. Er stellte drei Arten von Zellen fest: unspezialisierte Archäocyten, runde Dermalzellen und Choanocyten. Die letzteren sind insofern teilweise dedifferenziert, als sie den Kragen verloren haben, während sie die Geissel noch besitzen. Die Archäocyten sind sehr beweglich, die Dermalzellen nicht sehr lebhaft. Schon wenige Minuten nach Isolierung beginnen die Archäocyten sich zu bewegen und zwar unregelmässig. Vermöge der Klebrigkeit ihrer Plasmaoberfläche werden die im Umkreis einer Archäocyte liegenden übrigen Zellen aufgesammelt. Nach 24 Stunden ist eine Kugel gebildet, die mit einer dünnen Haut überzogen sein kann. Trifft die Archäocyte auf eine andere Zelle, so erfolgt meistens eine Richtungsänderung. Für den Aufbau des Schwammes ist also die Beweglichkeit der Archäocyten und der physikalische Zustand der Plasmaoberfläche entscheidend. Die Beweglichkeit wird allerdings durch die Anwesenheit artfremder Zellen (z. B. von *Cliona sulphurea*) gehemmt. Die Geschwindigkeit wechselt, sie nimmt vor allem ab bei Richtungsänderung. Eine Vereinigung von Zellen zweier verschiedener Arten gelang nie.

Der Aufbau des neuen Schwammes hängt nach Fauré-Fremiet (1925) von einem Gleichgewicht zwischen Beweglichkeit und Agglutinationstendenz ab, das durch eine Reihe äusserer Faktoren verschoben werden kann. Wie Galtsoff (1923) zeigen konnte, geht die Beweglichkeit nach etwa 170 Minuten in Ruhe über. Nach etwa einer Stunde sind Ansammlungen zustande gekommen, deren Abstände nicht mehr überbrückt werden. Sie werden aber dadurch noch vergrössert, dass die Schwammanlage in der Masse, wie die Histogenese einsetzt, sich noch weiter konzentriert. Bestimmt werden die Veränderungen auch durch Temperatur und die Anwesenheit bestimmter Kationen.

In einer weiteren Arbeit hat Galtsoff (1925 b) die Histogenese von *Microciona prolifera* geschildert. Er unterscheidet eine grössere Anzahl dissoziierter Zellsorten, nämlich Archäocyten, Pinacocyten, Desmacyten, Collencyten, Choanocyten, Skleroblasten, Spongoblasten und Gonocyten. Zu einem vollständigen Regenerat gehören etwa 2000 Zellen, die von den mit einer Kriechgeschwindigkeit von $0.6-3.5 \mu$ in der Minute umherwandernden Archäocyten gesammelt, wohl aber nicht nur zum Aufbau verwendet werden. Nach etwa drei Wochen ist mit dem Durchbruch des Osculums der Aufbau des Schwammes beendet. Notwendig zum Neuaufbau sind nach Galtsoff ausser den Archäocyten die Pinacocyten, die den Dermalzellen anderer Autoren entsprechen. Diese sollen auch Geisselzellen neu bilden. Eine Entdifferenzierung der dissoziierten Zellen konnte Galtsoff nicht nachweisen. Die Abstammung der differenzierten Zellen von den undifferenzierten gibt die Tabelle I wieder.

Tabelle I. Die Rolle der dissoziierten Zellen bei der Regeneration
(nach Galtsoff, 1925 b).



Später hat Galtsoff (1929) noch eine grössere Anzahl Kiesel- und Hornschwämme untersucht, die in verschiedener Schnelligkeit sich vereinigen konnten. Nach 12-24 Stunden lösten sich die jungen Schwammanlagen vielfach von der Unterlage los. Die Vereinigung der artgleichen Zellen wurde durch artfremde Zellen nicht nur gehemmt, sondern eine solche Durchmischung führt sogar zur Cytolyse und Agglutination der Zellen. Dies wird, wie nachzuweisen war, durch einen in Seewasser löslichen Stoff ausgelöst.

Eine sehr gründliche Arbeit über diesen Fragenkomplex stammt von Wilson u. Penney (1930). Auch sie benutzten *Microciona prolifera* zu ihren Untersuchungen, einen Schwamm, der an der ganzen atlantischen Küste Amerikas vorkommt. Die Hauptfrage, die die Autoren zu beantworten trachten, lautet: Machen die isolierten Zellen wirklich eine Dedifferenzierung durch, auf die eine Neudifferenzierung in die verschiedenen Gewebearten folgt, verlieren diese Zellen also ihre Individualität; oder findet nur eine zeitweise Entdifferenzierung statt, nach der sie zu ihrer ursprünglichen Zellform zurückkehren? Oder treten überhaupt undifferenzierte totipotente Zellen auf, die den neuen Schwamm aufbauen?

Isoliert man durch Mazeration Zellen in einem normalen Schwamm, so lässt sich eine Reihe von Zellarten, nämlich Zellen mit Nucleolus (Nucleoluszellen), Zellen ohne Nucleolus (graue Zellen), Stäbchenzellen, Kugelzellen, Faserzellen, Skleroblasten, Spongioblasten und Keimzellen unterscheiden. Sie alle gehören dem Mesenchym an. Dazu kommen noch die Kragenzellen und "epitheliale Syncytien", von früheren Autoren als Pinacocyten bezeichnet.

In dem durch Gaze gepressten Zellhaufen dagegen findet man: die Nucleoluszellen, die den Archäocyten Galtsoffs entsprechen und die grauen Zellen, daneben eine Anzahl der höher differenzierten Mesenchymzellen, darunter auch früher von Wilson fälschlich als Kragenzellen bezeichnete Elemente. Die echten Kragenzellen sind mit einer Geissel ausgerüstet, während der Kragen meist fehlt.

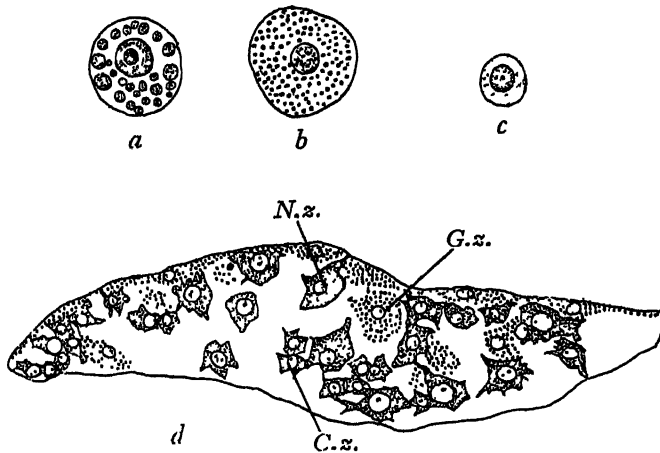


Fig. 1. Künstlich isolierte Schwammzellen. *a*, Nucleoluszelle; *b*, graue Zelle; *c*, Collarzelle, 1500 \times ; *d*, Paraffinschnitt, eine Stunde nach Durchpressen des Gewebes. Aussen graue Zellen, innen kleine Collar- und grosse Nucleoluszellen, 1250 \times (nach Wilson und Penney, 1930). *C.z.* Collarzelle, *G.z.* graue Zelle, *N.z.* Nucleoluszelle.

Nur die Nucleoluszellen, die grauen und die Kragenzellen bauen den neuen Schwamm auf (Fig. 1). Die Syncytien der Epidermis und der Kanäle sollen nach Ansicht der Autoren nicht zerfallen und die von Galtsoff als Pinacocyten bezeichneten Zellen sollen Kragenzellen sein. Vielleicht entsprechen den ersteren aber doch die grauen Zellen, denn bei der Neubildung des Schwammes bilden die grauen Zellen die Epidermis und die Auskleidung der Kanäle. Die Syncytienbildung der Epidermis ist vielleicht eine Folge der technischen Behandlung (Mazeration). Eine chemotaktische Wirkung der Zellen auf einander wird, wie von Galtsoff, so auch von diesen Autoren abgelehnt. Immerhin scheint doch eine stoffliche Beeinflussung dabei wirksam zu sein, wie aus den Bewegungen der isolierten Zellen, der Agglutinationserscheinung usw. zu erkennen ist.

Die Kragenzellen verharren in einem etwas undifferenzierten Zustande, sie bilden auch dann wieder unter Vermehrung die Kragenzellen des neuen Schwammes. Alle anderen höher spezialisierten Zellen des Mesenchyms mit Ausnahme der

Keimzellen werden aus unspezialisierten Mesenchymzellen neu gebildet. So ähnelt also der Neuaufbau des Schwammes weitgehend dem Regenerationsvorgang. Eine Ausnahme machen nur die Kragenzellen, die im Zustande der Dissoziation zeitweise sich dedifferenzieren und so die Möglichkeit haben, sich zu Kragengeisselzellen von neuem zu differenzieren.

Auf anderen Wegen ist E. Fauré-Fremiet (1931) zu ähnlichen Resultaten gekommen. Er fand bei einer histologischen Untersuchung von *Ficulina ficus*, einem Kieselschwamm, an Schnitten und Objektträgerkulturen Archäocyten und kernkörperlose Zellen, ausserdem aber sog. fuchsinophile Zellen, die den grauen Zellen Wilsons u. Penneys entsprechen sollen und den Desmacyten Galtsoffs. Diese schnell beweglichen Zellen sollen den ganzen Schwamm durchwandern.

Die Epidermis und die Auskleidung der Kanäle sollen nicht auf Syncytien zurückgehen, wie Wilson u. Penney angeben, sondern auf individualisierte Zellen.

In einer weiteren Arbeit hat derselbe Verfasser (1932a) das Schicksal dissoziierter Zellen verfolgt. Vier Zellarten bauen den neuen Schwamm auf: Choanocyten, Archäocyten, Collencyten und fuchsinophile Zellen. Sie werden zunächst durch Zufall durcheinander gelagert. Die thigmotaktischen Archäocyten breiten sich auf der Unterlage aus. Im Inneren kommt es zu Cytolyse und Zellvermehrung. Dann bilden die Collencyten ein lockeres Mesenchym, die Archäocyten Stränge spindelförmiger Zellen und die Fuchsinophilen wandern durch den Schwamm. Die Choanocyten dagegen werden aus dem Mutterschwamm übernommen und vereinigen sich zu unregelmässigen Massen, die später zu Hohlkugeln und schliesslich zu Geisselkammern werden, getrennt durch die Archäocytenstreifen. Die Pinacocyten der äusseren Oberfläche sollen aus den Collencyten hervorgehen, ebenso die Auskleidung der Kanäle.

In einer dritten Untersuchung hat Fauré-Fremiet (1932b) das Verhalten der isolierten Zellen mit solchen in Gewebekultur verglichen. Das organotypische Verhalten der Zellaggregate wird durch das Nebeneinander verschiedener Zellarten garantiert. Werden etwa durch Strahlenwirkung die empfindlichen Choanocyten ausgeschaltet, so ist eine allgemeine Involution die Folge, da eine Neubildung der Choanocyten in diesem Stadium anscheinend unmöglich ist.

Auf ganz anderem Wege haben aber auch Untersuchungen über die sog. Reduktionskörper bei Schwämmen und die Untersuchung der Gemmulac, die wohl ähnlich wie Reduktionskörper zu deuten sind, Klärung der behandelten Fragen gebracht. Es sei hier auf die Arbeit von K. Müller (1911b) hingewiesen, der diese Reduktionsvorgänge bei den Spongillidae untersuchte. Reduktionskörper sind kugelige Gebilde, die keine resistente Hülle wie die Gemmulae besitzen und kleiner sind als die normalen Schwammindividuen. Im Inneren findet man nur Archäocyten, ev. daneben noch Dermalzellen.

Die Gemmulae von *Spongilla lacustris* L. und *Ephydatia* sind nun auf ihre Zusammensetzung neuerdings von Brien (1932) ausführlich untersucht worden.

Der Verfasser konnte nachweisen, dass der aus der Gemmula hervorgehende Schwamm lediglich aus Archäocyten aufgebaut wird. Er bezeichnet sie als deutoplasmatische Archäocyten, die im Inneren Einschlüsse von Glycoproteinen mit

sich führen. Diese Zellen werden durch mitotische Teilung zweikernig und später bei günstiger Temperatur vielkernig. Beim Ausschlüpfen der Zellen zerfallen die vielkernigen Archäocyten in einzellige und machen eine Reihe Teilungen durch, die den Furchungsteilungen des Eies entsprechen (Fig. 2). Sie führen zu einer Differenzierung der Zellen in Histoblasten, die zuerst eine geschlossene Masse

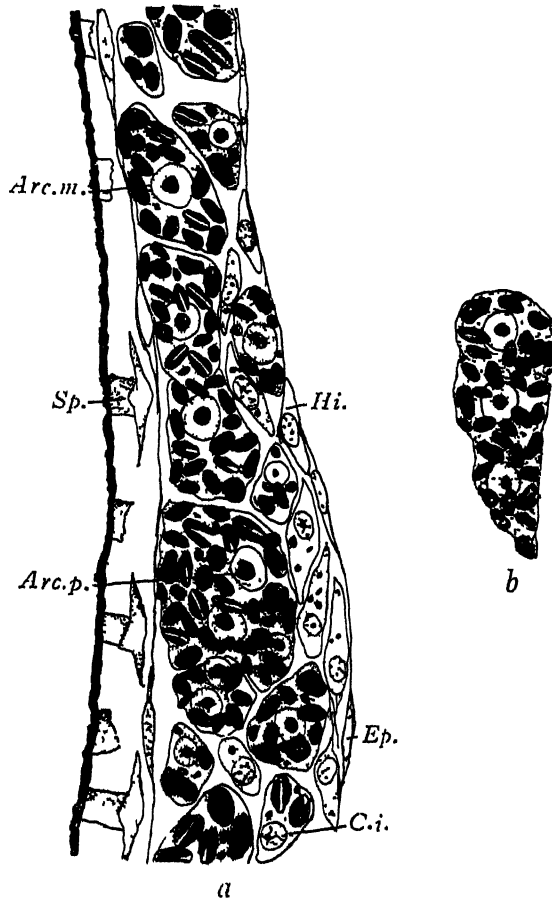


Fig. 2. Vielkernige Archäocyten. *a*, in der Histogenese der Gemmulae eines Süßwasserschwammes (*Spongilla lacustris*) (nach Brien, 1932). *Arc.m.* einkernige Archäocyten, *Arc.p.* vielkernige Archäocyten, *C.i.* Übergangszellen, *Ep.* Epidermiszellen, *Hi.* Histoblasten, *Sp.* Amphidiskien. *b*, eine einzelne vielkernige Archäocyte.

bilden, kein Deutoplasma mehr enthalten, bald aber regionenweise sich differenzieren: Die äusseren werden zu Epidermis, die inneren Lagen bilden Mesenchym usw. Diese Entwicklung aus der Gemmula entspricht also der Embryogenese, lediglich kompliziert durch die Einschaltung mehrerer Archäocytenstadien.

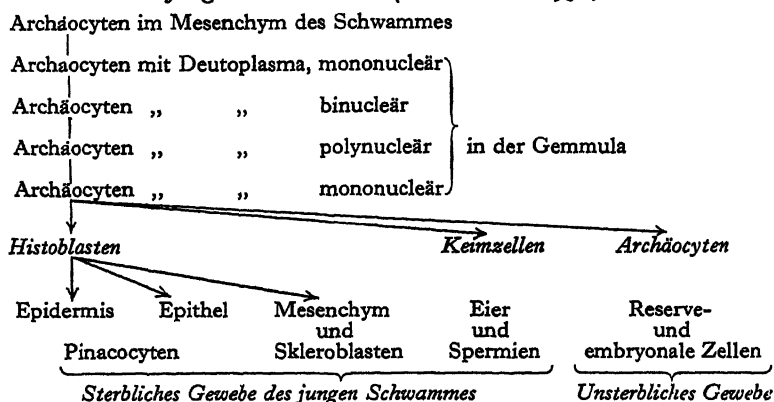
Das Verhältnis der Archäocyten zu den differenzierten Geweben und den restlichen undifferenzierten Archäocyten des ausgebildeten Schwammes gibt die Tabelle II wieder. Die Ausgangsformen dieser totipotenten Zellen in der Gemmula

sind aber durch die Einlagerung von Glycoproteinen verändert und damit für ihre Aufgabe vorbereitet. Sie sind die eigentlichen unsterblichen Zellen, denen gegenüber die somatischen Zellen wie die Keimzellen differenziert und deshalb sterblich sind.

Die Gastralzellen werden durch Phagocytose von den Archäocyten beseitigt.

Zusammenfassung. Bei den histogenetischen Prozessen im Schwammorganismus lassen sich zwei Modi unterscheiden. Den einen findet man bei der Reunion nach Dissoziation: Der neue Schwamm wird aus zwei oder drei Zellsorten aufgebaut, die als Archäocyten und graue Zellen bezeichnet werden, falls diese letzteren nicht nur eine besondere Form der Archäocyten darstellen. Dagegen bleiben die Choanocyten nach geringfügiger Dedifferenzierung die Grundlage für den Aufbau des Darmes. Durch Bestrahlung können die Choanocyten so geschädigt werden,

Tabelle II. *Die Bedeutung der Archäocyten für den Aufbau des jungen Schwammes (nach Brien, 1932).*



dass der Neuaufbau des Schwammes unmöglich wird. Diese entodermalen Zellen sind also nicht durch andere ersetzbar.

Im Gegensatz dazu besteht der andere Modus der Histogenese in völliger Neubildung aus den Gemmulae und den Reduktionskörpern lediglich durch die Archäocyten.

III. COELENTERATA.

In dieser Gruppe wurden die meisten Untersuchungen an den Gattungen *Chlorohydra*, *Hydra* und *Pelmatohydra* angestellt. Schon Nussbaum (1887) und nach ihm besonders Schulze (1918) hatten den interstitiellen Zellen (I-Zellen) die Aufgabe des Neuaufbaues der Regenerate zuerkannt. Darüber ist in den letzten Jahren eine lebhafte Diskussion entbrannt.

I-Zellen sind nach Kleinenberg (1872) Zellen, die zwischen den Epithelmuskelzellen liegen—daher der Name interstitielle Zellen—und nicht an der Stützlamelle befestigt sind. Sie besitzen einen verhältnismässig grossen Kern mit sehr geringem, dunkel sich färbenden Plasma.

Nach einer kurzen Mitteilung von Rowley (1902), die mangels bildlicher

Belege nicht als entscheidend angesehen werden kann, hat zuerst Mattes (1925) die Frage nach der Herkunft des Baumaterials bei *Pelmatohydra*, *Chlorohydra* und *Hydra* wieder aufgeworfen. Er schildert die Überbrückung einer durch Eingriff gesetzten Lücke in der Körperwand durch sich abflachende Ektodermzellen, manchmal auch durch Ektoderm- und Entodermzellen. Es sind also bei der Wundheilung zunächst differenzierte Zellen am Werke. Ektoderm- und Entodermzellen bleiben geschieden, wenn auch die Grenze zwischen ihnen in Form der Stützlamelle erst später wieder deutlich wird. Auch die Muskelfibrillen differenzieren sich in den bei der Wundheilung tätigen Zellen erst später heraus.

Mitosen treten in der Nähe der Wunde nicht auf, sondern man sieht sie an fern gelegenen Stellen des Hydrakörpers und zwar hauptsächlich im Ektoderm, weniger im Entoderm, nur in jungen Zellen. Später sind die Mitosen diffus verteilt. Man muss also annehmen, dass nach Wundsetzung die I-Zellen auf die Wunde zuwandern und Neubildung der abgewanderten I-Zellen fern von der Wunde erfolgt. Dort erscheinen auch die I-Zellen verändert: ihr Chromatin ist aufgelockert und die Zellen vergrössert. Nach 4–6 Tagen ist der Wundverschluss meist beendet. Der Aufbau der Gewebe erfolgt also zunächst mit Hilfe der differenzierten Nachbargewebe, die zu diesem Zwecke vielleicht eine schwache Dedifferenzierung durchmachen, des weiteren unter Zuhilfenahme der zuwandernden I-Zellen. Ähnliches stellte Godlewski (1904) für die Regeneration von *Tubularia mesembryanthemum* fest.

Kanajew (1926) hat diesen Wundheilungsprozess bei *Pelmatohydra* bestätigt. Er fand die Entodermzellen bei der Schliessung der Wunde vielfach in lebhafterer Tätigkeit als die Ektodermzellen. Im Ektoderm fehlten Zellgrenzen. Nach der Wundheilung rücken die I-Zellen im Ektoderm des Wundrandes heran. Sie sind kenntlich durch ihr dunkles Plasma und den Kern mit körnigem Inhalt. Dies war etwa 24 Stunden nach der Operation zu beobachten. Gruppenweise erfolgt nun die Bildung der Nesselkapseln. Mitosen sind aber nirgends häufiger als in normalen Tieren. Als Besonderheit stellte Kanajew fest, dass I-Zellen auch durch die Stützlamelle in das Entoderm wandern und dort wahrscheinlich neue Entodermzellen bilden. Nach Kanajew besteht die Regeneration der Hydren hauptsächlich in der Umbildung vorhandener I-Zellen. Dafür spricht auch die Armut an I-Zellen in regenerierten Hydren.

In gleicher Weise geht Issajew (1926) bei seinen Betrachtungen von der Annahme aus, dass die I-Zellen die Grundlage für den Aufbau des Hydrenkörpers darstellen.

In einer zweiten Arbeit hat Kanajew (1926 b) einzelne histologische Tatsachen zur Ergänzung mitgeteilt. Auch hier wurde wieder der Wundverschluss durch sich streckende Entodermzellen beobachtet (*Pelmatohydra*). Nach 12 Stunden ist das Entoderm der Wundregion dunkler gefärbt, das Plasma reich mit Nährstoffen gefüllt, die Zellen dichter gelagert. Kanajew vermutet eine Zuwanderung von I-Zellen in diese Region. Es besteht hier eine Parallele zur Knospenbildung, die auch an einer Anhäufungsstelle von I-Zellen erfolgt. Die histologische Untersuchung dieser Zellansammlungen liess viele junge Entodermzellen erkennen, die wohl aus I-Zellen hervorgegangen sind.

Mit Hilfe der Röntgenstrahlen haben Zawarzin (1929) und Strelin (1929a) Aufschluss zu gewinnen versucht über die Bedeutung der einzelnen Zellarten bei regenerativen Vorgängen. Sie fanden, dass diese Strahlen sowohl stimulierend wie schädigend wirken können. Die stimulierende Wirkung kann sich sekundär in stärkerer Regeneration zu erkennen geben. Operierte Hydren können dann stärkere Dosen von Röntgenstrahlen vertragen als nicht operierte. Die histologische Untersuchung der bestrahlten Hydren durch Strelin ergab nun, dass stark bestrahlte Hydren keine I-Zellen mehr besaßen, die sich infolge der Strahlenwirkung nicht mehr vermehrten. Eine gewisse Degeneration der Hydren ist die Folge. Die Wiederherstellung der Teilungsfunktion dauert bei den I-Zellen länger als bei den differenzierten Zellen. Die grössere Empfindlichkeit der Sommerhydren gegenüber den Herbsthydren, von der Zawarzin berichtet, ist vielleicht auf die zahlreicheren I-Elemente unter besserer Ernährung im Sommer zu erklären. In dem Verhalten der differenzierten Zellen den Röntgenstrahlen gegenüber spielen die Entodermzellen eine besondere Rolle, denn sie bilden sich bei Anwendung nicht zu starker Strahledosen zu dem eigenartigen Epithel der Mundscheibe um, bei völligem Fehlen von I-Zellen. Diese Versuche zeigen also, dass die I-Zellen als undifferenzierte Zellen durch Röntgenstrahlen am meisten geschädigt werden, was zu ihrem Untergang oder zu relativ später Erholung führt. Relativ wandlungsfähig scheinen die Entodermzellen zu sein.

Strelin (1929b) beschreibt die Umwandlung der interstitiellen Zellen in Epithelmuskelzellen, ein Vorgang, der nach Röntgenbestrahlung infolge des fehlenden Nachschubs an I-Zellen sich besonders gut beobachten lässt. Dabei treten Mitosen gruppenweise in I-Zellen und jungen Epithelzellen auf. Wie Zawarzin gibt auch der Autor an, dass die Sekretzellen der Mundscheibe sich aus den Drüsenzellen des Entoderms bilden können.

Im Gegensatz zu allen bisherigen Angaben und zu eigenen früheren Mitteilungen hat Kanajew (1930) bei *Pelmatohydra oligactis* nachzuweisen versucht, dass die I-Zellen bei der Regeneration nur eine geringfügige Rolle spielen. Die differenzierten Zellen, die die Wundheilung vollbringen, werden später kompakter und enthalten Einschlüsse. Kanajew untersuchte Mundregion und Fussregion und fand wenige oder keine I-Zellen bei ihrer Regeneration beteiligt, auch bei der Knospenbildung fand er nicht so reichlich I-Zellen, wie er vermutet hatte. Er nimmt im Entoderm Regeneration aus differenzierten Zellen an. Trotz zugestandenen geringen Untersuchungsmaterials vertritt der Autor seine Behauptung, wenn er auch einige Wanderzellen in der Stützlamelle beobachtete. Er gibt aber nicht an, in welchem Ernährungszustand seine Tiere waren. Kanajew sieht vielmehr in dem Hydrakörper gewisse Zentren physiologischer Art, die sich Nachbargebiete subordinieren (Organisationszentrum).

Wie zu erwarten, hat diese Ansicht Kanajews zu Nachuntersuchungen Anlass gegeben. So hat Honczek (1934) grosse Teile des Entoderms der *Hydra* abgeschabt und die Regeneration verfolgte. Sie erfolgte, wie anzunehmen war, auf dem Wege der normalen Wundheilung durch Streckung der übrigen Entodermzellen, denn man sah dort keine Mitosen. Infolge der nur noch wenigen vorhandenen Entodermzellen

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wurde das Ektoderm stark gefaltet, z. T. Zellen von ihm abgestossen. Bis zum 4. Tag wurden die Tiere beobachtet. Was dann geschah, wurde nicht verfolgt. Auch hier ist der Lebenszustand der *Hydra* besonders nach so tiefgreifenden Eingriffen zu berücksichtigen. Der Verfasser sah spindelförmige Zellen mit drüsigem Inhalt im Entoderm, deren Herkunft er nicht nachweisen konnte.

Dass übrigens auch im Entoderm reichlich Mitosen in den epithelio-muskulären Zellen vorkommen, besonders in der Zeit, wo die geschlechtliche wie die ungeschlechtliche Fortpflanzung ruht, hat McConnell (1932) gezeigt.

Über den Aufbau der Knospe sind wir durch eine Reihe von Arbeiten unterrichtet worden.

Schon A. Lang (1892) nahm an, dass die Knospung von *Hydra* von den I-Zellen ihren Ausgang nimmt. Tannreuther (1908-9) sah ebenfalls zuerst eine Vermehrung der I-Zellen. Dagegen wandern nicht, wie Lang meinte, Ektodermzellen ins Entoderm, sondern eine Ausbuchtung beider Zellschichten auf Grund von

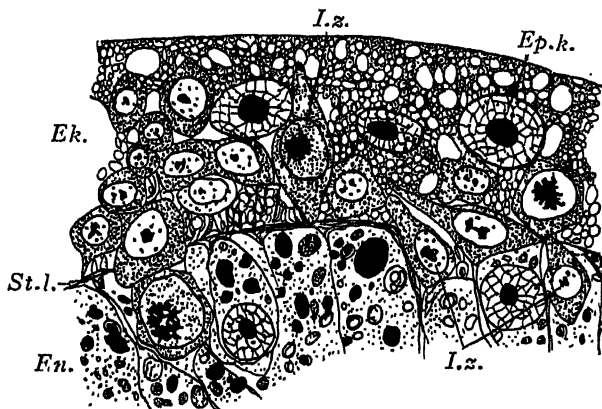


Fig. 3. Teil der Körperwand von *Hydra* spec. zu Beginn der Knospenbildung (nach Hadzi, 1910). Ek. Ektoderm, En. Entoderm, Ep.k. Epidermiskern, I.z. interstitielle Zellen, St.l. Stützlamelle.

Zellvermehrung rechts und links der zukünftigen Knospenspitze ist die Grundlage der Knospenbildung. Im Ektoderm vermehren sich die I-Zellen, im Entoderm überwiegend die differenzierten Darmzellen. Die Schnelligkeit der Knospung ist von der Futtermenge abhängig, und Hunger kann zur Rückbildung der Knospe führen. Hadzi (1910) gibt für *Hydra fusca* an, dass der Beginn der Knospenbildung in Ansammlung von I-Zellen zu sehen ist, die aus der Basis des Ektoderms stammen und deutliche Stadien der Bewegungen zeigen. Sie häufen sich im Ektoderm an, während das Entoderm zunächst unverändert bleibt. Durch Umbildung von indifferenten zu differenzierten Zellen kommt eine Oberflächenvergrößerung des Ektoderms zustande. Eine entsprechende Ausdehnung des Entoderms wird durch Überwanderung von I-Zellen durch die Stützlamelle hindurch in das Entoderm erzielt (Fig. 3). Es werden also in beiden Zellschichten die Mutterzellen mit Knospenzellen durchsetzt. In die Tentakel dagegen sollen indifferente Zellen nur aus dem Entoderm einwandern.

v. Gelei (1925) dagegen fand zu Beginn der Sprossbildung die Zellen beider

Körperschichten z. T. in mitotischer Teilung. Dazu treten in beiden Schichten noch I-Zellen. Gegenüber Hadzi betont v. Gelei, dass anfänglich auch das Entoderm sich verdickt ohne Zuwanderung von I-Zellen. Bei *Hydra grisea* beobachtete er aber auch Mitosen im Entoderm. Die Verdickung der Schichten zu Beginn der Sprossbildung führt v. Gelei nicht so sehr auf Ansammlung von I-Zellen, als auf einen Zustrom von Nährstoffen zurück.

Die I-Zellen sind dauernd teilungsfähig und stellen die Reserve an Zellmaterial für *Hydra* dar: nur bei schlechter Ernährung kann der Bestand an I-Zellen soweit zurückgehen, dass die Neubildung differenzierter Zellen nicht mehr möglich ist (Schlottke, 1930).

Papenfuss (1934) konnte nachweisen, dass eine Reunion nach Dissoziation einzelner Zellen von *Hydra* nicht stattfindet. Nur einzelne Gewebestücke kommen zur Vereinigung. Dabei geht die Initiative von dem Entoderm aus, das protoplasmatische Fortsätze aussendet. Das Ektoderm wölbt sich über die Entodermzellen und bildet mit ihnen einen Cylinder. Überzahl an einer Zellart wird regulativ ausgeglichen. Die Beteiligung der I-Zellen ist ungeklärt.

Ausser diesen Untersuchungen an Hydren liegen nur sehr wenige Versuche vor, den Aufbau des Regenerates bei Coelenteraten zu verfolgen. Schon oben waren Mitteilungen von Godlewski (1904) erwähnt worden, die für unsere Frage nur insofern von Interesse sind, als die Entodermzellen der untersuchten *Tubularia mesembryanthemum*, nach Setzung einer Wunde am Darm, das Bestreben haben sich zu sammeln und zwar in mehreren Schichten, innerhalb derer ein neues Lumen gebildet wird. In diesem Kanal zirkulieren Körnchen, die von degenerierenden Darmzellen stammen und nach Ansicht des Autors formative Stoffe führen, die zum Aufbau des definitiven Darmes notwendig sind. Über den Aufbau der ektodermalen Schicht finden sich keine Angaben.

Als weitere sehr wichtige Arbeit ist noch die von H. V. Wilson (1911) zu nennen, in der versucht wird, Zellen von Hydroiden und Alcyonarien zu dissoziieren und die Histogenese zu verfolgen. Sowohl für *Eudendrium* wie für *Pennaria* schildert Wilson den Aufbau des Polypen aus den differenzierten Zellen, die wohl nur eine vorübergehende Dedifferenzierung durchmachen, wobei sie Kugelform annehmen. Sehr bald ordnen sie sich wieder in zwei Schichten an und bilden so den neuen Polypen. Wie weit die Dedifferenzierung geht und ob daneben überhaupt noch eine dritte Zellsorte vorkommt, ist fraglich. Das gleiche Resultat wurde bei der Alcyonarie *Leptogorgia* erzielt. Diese Untersuchungen bedürfen dringend einer Nachprüfung.

Zusammenfassung. Die untersuchten Formen der drei Hydrengattungen lassen die grosse Bedeutung der I-Zellen erkennen, die im Regenerat das Ektoderm mit seinen Differenzierungen aufbauen, aber auch in das Entoderm hinüberwandern und dort beim Aufbau dieses Gewebes helfen. Allerdings scheint das Entoderm weniger differenziert zu sein im Verhältnis zum Ektoderm, sodass es in der Lage ist von sich aus diese Körperschicht weitgehend zu regenerieren, wie das Vorkommen von Mitosen zeigt. Auch können Entodermzellen die besonders differenzierten Zellen der Mundscheibe bilden.

Die von Kanajew (1930) behauptete geringe Bedeutung der I-Zellen bedarf der Nachprüfung.

Auch bei der Bildung der Knospen und der Gonaden wirken die I-Zellen entscheidend mit. Bei Bestrahlung der Hydren mit Röntgenstrahlen erweisen sich die I-Zellen als besonders empfindlich. Ihre Ausschaltung durch die Strahlen lässt auch die regenerativen Prozesse zum Stillstand kommen.

IV. TURBELLARIA.

Bei der Untersuchung der Regenerationsvorgänge der Turbellarien hat man erst spät die zellulären Vorgänge, die sich dabei abspielen, in den Kreis der Betrachtung gezogen. Keller (1894) nannte die bei der Regeneration tätigen Zellen "Stammzellen", die im Parenchym liegen. Ausser dem Regenerat geben sie auch den Geschlechtsorganen den Ursprung.

Die erste Arbeit, die ausführlicher die Histogenese des Regenerates verfolgt, ist die von Stevens (1907). Sie stellte bei drei Arten der Gattung *Planaria* fest, dass nach der Wundsetzung die Wundränder gegen einander bewegt werden und Ektodermzellen durch ihre Formveränderung den ersten Verschluss der Wunde bilden, indem sie sich in tangentialer Richtung strecken. Später wandern dann auch Parenchymzellen in das Wundektoderm ein. Wie Versuche erkennen lassen, werden die Darmteile ebenfalls von Parenchymzellen gebildet, meist in unmittelbarem Zusammenhang mit dem alten Entoderm.

Lang (1912, 1913) hat dargelegt, dass die Parenchymzellen zum grössten Teile sog. Übergangszellen sind, d. h. entdifferenzierte Zellen, die einer Neudifferenzierung entgegen gehen. Er leugnet überhaupt, dass embryonale Stammzellen vorkommen. Die Regeneration des Epithels schildert Lang (1913) schon so, wie später andere Autoren auch (s. u.). Dabei soll die Zellvermehrung auf amitotischem Wege eine bedeutende Rolle spielen. Von Curtis (1902) wurde angegeben, dass das Mesenchym die Wunde schliesse.

Die grundlegende Arbeit für die Frage der Herkunft des Materials ist die Arbeit von Bartsch (1923 a, b). Er konnte die ausschlaggebende Rolle der Parenchym- (= Mesenchym-)zellen bei allen Regenerationsvorgängen erweisen. Sein Material waren die Gattungen *Planaria*, *Dendrocoelum* und *Polycelis*. Bartsch sah, dass die letzten der unversehrten Epidermiszellen nach dem Eingriff sich seitlich über die Wunde neigen und sich dabei verlängern. Zellwandungen schwinden, die Kerne liegen regellos durcheinander. Das ganze Syncytium fliesst wie eine Amöbe über die Wunde (Fig. 4a). Dieses Häutchen ist im ganzen Bereich der Wunde sehr verschieden an Dicke, da es aus einem Netzwerk amöboider Zellen entstanden ist. Das ist der Zustand des Häutchens nach etwa einer Stunde. Die Wimpern dieser Epithelzellen bleiben erhalten, nur stehen sie in grossen Abständen. Unter diese oberste Lage von Zellen lagern sich Regenerationszellen, die aus dem Parenchym stammen. Durch den von ihnen ausgehenden Wachstumsdruck sollen sie die Epidermis vor sich her wölben. Eine Einwanderung von Parenchymzellen in das Epithel soll nicht stattfinden, wenn Bartsch auch annimmt, dass passiv solche

Zellen in das Epithel gedrückt werden, wie er feststellen konnte (Fig. 4*b*). Bartsch hält die Regenerationszellen für entdifferenzierte Mesenchymzellen, die in diesem Stadium omnipotent sind. Sie zeigen die übliche amöboide Form, die wandernde Zellen erkennen lassen. Im Epithel wurden niemals Mitosen beobachtet.

Das Darmsystem, der Pharynx ebenso wie der Mitteldarm, wird bei Regeneration durch Ansammlungen von solchen Mesenchymzellen gebildet, die zwischen sich ein Lumen entstehen lassen (Fig. 4*c*). Ebenso werden Rhabditenbildungszellen und Muskulatur aus den Mesenchymzellen (Regenerationszellen) gebildet. Der Neuaufbau der Regenerate ist also im wesentlichen ein Restitutionsvorgang. Bartsch möchte die Zellen als Restitutionszellen bezeichnet haben.

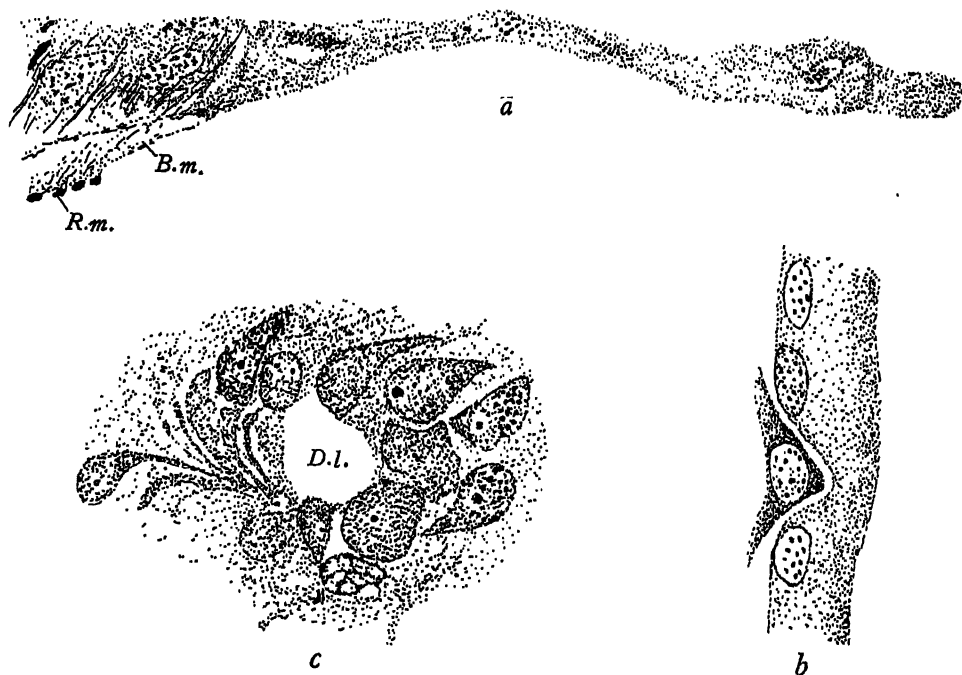


Fig. 4. *Planaria* spec. a, Wundheilungshäutchen, 4 Stunden nach Wundsetzung. b, einwandernde Regenerationszelle. c, erste Anlage des Darmes im Regenerationskegel. Nach Bartsch, 1923. B.m. Basalmembran, D.l. Darmlumen, R.m. Ringmuskeln.

Später hat Kenk (1924) auch für den Kopulationsapparat die Regeneration durch diese Zellen bewiesen. Auch er hielt die "Übergangszellen" und Regenerationszellen für entdifferenzierte Mesenchymzellen.

Eine ebenfalls ausführliche Untersuchung, gleichfalls an Planarien, nahm Steinmann (1926) vor. Die erste Überdeckung der Wunde sieht er als Schleim an, nicht als Zellbelag. Danach kommt es wieder zu einer Vergrößerung der Wundfläche, die sich zuerst extrem zusammengezogen hatte. Innerhalb der ersten 24 Stunden legt sich sodann das Epithel in der oben beschriebenen Weise über die Wunde. Oft unterbleibt aber auch der Epithelverschluss und Regenerationszellen bauen das neue Epithel auf, wahrscheinlich dann, wenn die Wunde anfänglich sich

nicht genügend weit schliesst. Die Abstammung des Verschlussepithels vom alten Epithel erkennt man an der Anwesenheit von Rhabditen.

Auch Steinmann betrachtet die Regenerationszellen als dedifferenzierte Körperzellen. Sie treten schon kurz nach der Wundsetzung an der Stelle des Eingriffes auf, ohne dass Mitosen sichtbar werden. Geschlechtsorgane und andere Körperorgane werden abgebaut, durch Phagocytose und Zellwanderung kommt es zu einer Umordnung der Elemente, die alle auf die Wundfläche zuwandern. Die Wanderzellen sind meist spindelig gebaut und mit einem dunklen Plasmabezirk ausgestattet. Die Reihenfolge der Dedifferenzierungen ist so, dass zuerst und längere Zeit die Mesenchymzellen sich umwandeln, dann folgen von den Geschlechtsorganen zuerst die Dotterstöcke, zuletzt die Gonaden selbst, also erfolgt der Abbau in umgekehrter Reihenfolge der postembryonalen Entwicklung. Voll differenzierte Zellen zerfallen, degenerieren und werden als Nährstoffe an die Wundstelle transportiert. Die anderen Elemente dedifferenzieren sich und wandern selbständig auf die Wunde zu.

Curtis u. Schulze (1924) fanden, dass entsprechend der verschiedenen Regenerationsfähigkeit bei den Tricladenarten *Planaria maculata*, *Phagocata gracilis* und *Dendrocoelum lacteum* die Zahl der zur Verfügung stehenden Bildungszellen verschieden ist. Für die drei Arten wurden die Zahlen 70-45, 22 und 15 auf gleichem Raume festgestellt. Diese Regenerationszellen sind also die eigentlichen aktiven Elemente. Sie teilen sich auf mitotischem Wege.

Bei den Rhabdocölen, überwiegend bei *Mesostoma*-arten, hatt Fulinski (1922) nur Wundheilung festgestellt. Diese Reparation hängt aber weitgehend von der Grösse des Ausgangsstückes ab. Innerhalb dieser Gruppe ist die Neigung zur Regeneration bei den Hysterophoren grösser als bei den Lecithophoren.

Diese Frage hat einige Jahre später Hein (1928) wieder geprüft und zwar gerade bei den Gattungen *Dalyella*, *Typhloplana* und *Rhynchomesostoma*, die zu den Lecithophoren gehören.

Bei allen drei Gattungen war ebenfalls nur Wundheilung zu bemerken. Schon im normalen Epithel sieht man hin und wieder Ersatzzellen auftreten, deren Herkunft unbestimmt ist. Bei der Wundheilung nach einem am Hinterende des Tieres vorgenommenen Eingriff kommt es, wie schon Bartsch für Tricladen schilderte, zu einer Überdeckung der Wunde durch abgeflachte Zellen. Bei der weiteren Ausbildung des Häutchens zum normalen Epithel sollen mesenchymale Zellen einwandern. Aus beiden Anteilen wird dann das definitive Epithel aufgebaut. Das Wundhäutchen ist nach 2-4 Tagen entwickelt, nach 9-11 Tagen das neue Epithel hergestellt und nach 17-20 Tagen kommt der ganze Prozess zum Ende.

Die aufbauenden Zellen sieht die Verfn. als freie Bindegewebezellen an, die bei diesen Formen relativ spärlich sind. Diese Tatsache und die Beobachtung, dass eine Dedifferenzierung von Organen oder Organteilen bei den untersuchten Formen nicht vorkommt, gibt die Erklärung dafür, dass es bei den Rhabdocölen nur zur Wundheilung, nicht aber zu eigentlicher Regeneration kommt. Nur bei *Rhynchomesostoma* beobachtet man eine weitergehende Neubildung von Geweben, wofür aber die Gründe nicht festzustellen waren, da das Material zu gering war.

Interessante Erscheinungen am Regenerationsmaterial waren nun aber unter besonderen Bedingungen zu beobachten. So hielt J. W. Wilson (1926) *Planaria maculata* in isotonischer Ringerlösung. Es kam dann zu keinem Wundverschluss, sondern von zwei Zentren von Regenerationszellen aus wurden die zwei Hälften eines Kopfes angelegt und zum Schluss durch eine Brücke neuen Gewebes verbunden. Und so wurde von hinten nach vorn die Wunde geschlossen. Ebenso wird ein fehlendes Hinterende von zwei seitlichen Zentren aus aufgebaut. Daraus ergibt sich, dass Wundverschluss für den Regenerationsvorgang als solchen nicht notwendig ist und dass dabei keine Entdifferenzierung stattfindet, sondern neues Gewebe gebildet wird. Man könnte daraus schliessen—was der Autor nicht tut—dass Wundverschluss und Entdifferenzierungsvorgänge durch den Einbruch des Mediums in das Körperinnere in Gang gesetzt werden.

Curtis u. Hickman (1926) gehen von der Anschauung aus, dass die Regenerationsfähigkeit der Planarien von der Menge der zu Verfügung stehenden "formativen" Zellen abhängt. Wurden daher diese Zellen Röntgenstrahlen von einer gewissen Stärke ausgesetzt, so wurden sie zerstört und die Regeneration blieb aus, während die Kontrollen in 12 Tagen vollständig regenerierten. Ähnliche Resultate wurden mit Radium erzielt (entsprechende Versuche an dem Schwamme *Microciona prolifera* und dem Hydroidpolypen *Tubularia crocea* hatten gleichen Erfolg). Danach müssen die "formativen" Zellen der Planarien den embryonalen ähnlich sein.

Unter gewissen ungünstigen Bedingungen kommt es bei Planarien zur Bildung von Schleimcysten aus abgetrennten Hinterenden. Nach einigen Wochen bildet sich aus einer solchen Cyste wieder ein neues Tier. Alexander u. Price (1926) verfolgten die dabei eintretende Degeneration der Gewebe, die bis zu ihrer völligen Reduktion, d. h. bis zu einem granulären Plasma mit einigen Zellen und schlecht erkennbaren Kernen fortschreitet. Das Ektoderm allerdings bleibt bestehen, aber seine zelluläre Struktur ist auch nicht mehr erkennbar. Ob die in der Plasmamasse sichtbaren Zellen formative sind, war nicht zu entscheiden. Die Gesamtplasmamasse dient wahrscheinlich als Nährmaterial für den Aufbau der neuen Gewebe.

Als neuere Untersuchung über Strahlenwirkung ist die von Weigand (1930) zu nennen. Der Verfasser verwendete *Planaria* und vor allem *Polycelis nigra*. Als Strahlenquelle diente ein Radiumbromidpräparat. Schon nach zwei Stunden zeigen sich deutliche Unterschiede zwischen bestrahlten und unbestrahlten Tieren, da bei den ersteren die Mitosen im Parenchym selten werden oder ganz fehlen. Nach längerer Zeit kommt es zu abnormen Teilungen im Parenchym. Hemmung der Regeneration tritt schon nach einer Bestrahlung von 10–15 Minuten ein, Degenerationserscheinungen am differenzierten Gewebe scheinen von den Regenerationszellen ihren Ausgang zu nehmen, die so frühzeitig geschädigt wurden, dass sie nicht mehr an den Regenerationsort wandern konnten.

Es bestätigt sich also das Gesetz von Bergonié u. Tribondeau, dass die undifferenziertesten Zellen am meisten geschädigt werden. Stärkere Bestrahlung kann die gesamte Menge der Regenerationszellen treffen. Dagegen scheint eine Stimulationswirkung zu fehlen, die bei den Hydren noch eine Rolle spielt.

Bestrahlung nach Operation ist weniger wirkungsvoll, weil die Strahlen schon auf Differenzierungsvorgänge in der Regenerationsknospe treffen, also nicht mehr so wirksam sein können.

van Cleave (1934) setzte *Stenostomum* grossen Dosen von unfiltrierten Röntgenstrahlen aus. Diese Form erwies sich als relativ widerstandsfähig: 20–30,000 R-Einheiten hinderten die Regeneration nicht. Dazu standen in Gegensatz Kontrollversuche an der Oligochätengattung *Pristina*, bei der bei gleicher Dosis die Regeneration des Kopfes unterblieb und bei *Dero*, die bei dieser Dosis der Cytolyse verfiel.

Auf einem anderen Wege hat Murray (1931) versucht Einblick in das Verhalten des Parenchyms zu gewinnen. Dieses wurde nach steriler Behandlung der Versuchstiere (*Planaria dorotocephala*) in einer Nährlösung gehalten, in der Gewebe von Tricladen sich 6 Wochen hält. Nach mehrtägiger Kultivierung nahmen die Zellen ein deutlich polarisierten Aussehen an, und sie können Nervenzellen so ähnlich werden, dass man schliessen kann, dass die Regeneration von Kopfbenden wahrscheinlich unter Zuhilfenahme solcher Parenchymzellen erfolgt.

In einer ausführlicheren Arbeit betont Murray (1927), dass die Ektodermzellen *in vitro* sehr unempfindlich sind und besonders lange leben, auch die stärksten Schwankungen der Konzentration des Mediums ertragen. Das Epithel reguliert ja auch im intakten Organismus die Differenzen des osmotischen Druckes zwischen Innen und Aussen.

In neuester Zeit haben Curtis u. Schulze (1934) die beiden Planariengattungen *Planaria* und *Procotyla* bezüglich ihres Regenerationsvermögens mit einander verglichen. *Planaria* hat eine sehr grosse Regenerationsfähigkeit, *Procotyla* eine sehr geringe. *Planaria* regeneriert noch, wenn das Tier in 25 Stücke zerlegt wird, während *Procotyla* derartig zerlegt abstirbt. So regeneriert das letzte Drittel (hinter dem Pharynx) bei *Procotyla* nicht mehr, während die entsprechenden Stücke bei der Gattung *Planaria* sehr gut regenerieren. Die präpharyngeale und die Pharynxregion selbst regenerieren allerdings bei *Procotyla* besser, aber diese Fähigkeit ist doch viel geringer als die der entsprechenden Regionen von *Planaria*. Diese Unterschiede finden ihre Erklärung in der Menge der zur Verfügung stehenden "formativen" Zellen (Fig. 5). Bei *Planaria* sind auf der gleichen Fläche im histologischen Bilde viel mehr solcher Zellen zu sehen als bei *Procotyla*. Dabei ist es gleichgültig ob ungeschlechtliche oder Geschlechtsindividuen untersucht wurden. Bei *Procotyla* stammen auch die Keimzellen von diesen formativen Zellen ab.

Da keinerlei Dedifferenzierungsvorgänge beobachtet wurden, schliessen die Verfasser, dass diese Bildungszellen Embryonalzellen ("embryonic stock") sind und nicht dedifferenzierte Mesenchymzellen. Die Anzahl der zur Verfügung stehenden formativen Zellen und Regenerationsfähigkeit entsprechen sich also. Die Herkunft der embryonalen Zellen und ihre Beziehungen zu den übrigen Geweben des Planarienkörpers ist in der Tabelle III dargestellt.

Ähnlich wie der Regenerationsvorgang muss aber auch die ungeschlechtliche Fortpflanzung der Turbellarien betrachtet werden. Nach Vandel (1921 a) sind es auch hier die jungen widerstandsfähigen Tiere, die die Teilung zeigen (*Polycelis cornuta*), nicht aber etwa degenerierte Tiere, wie andere Autoren vermuteten.

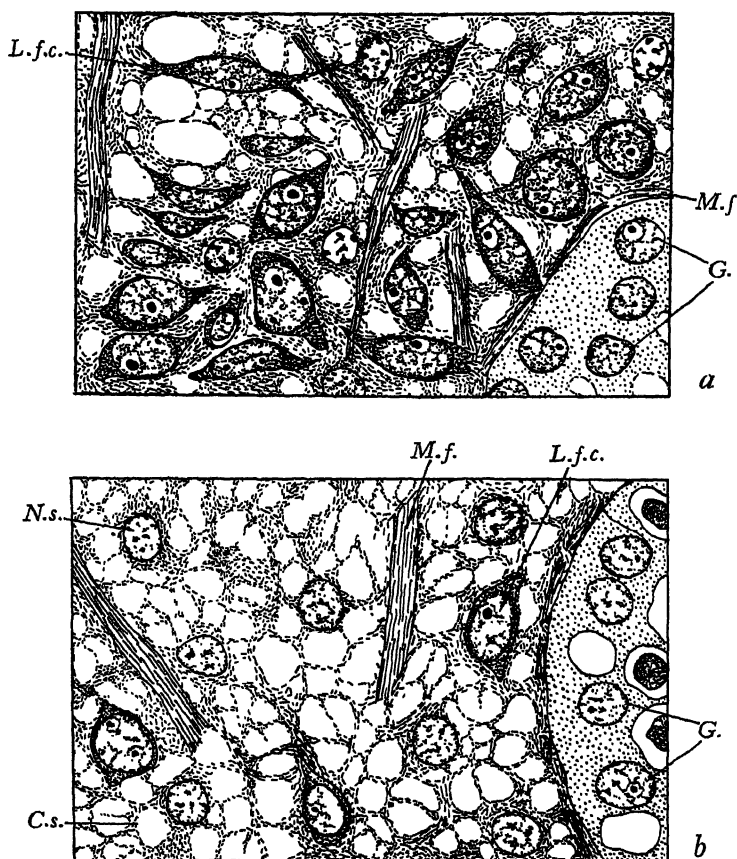
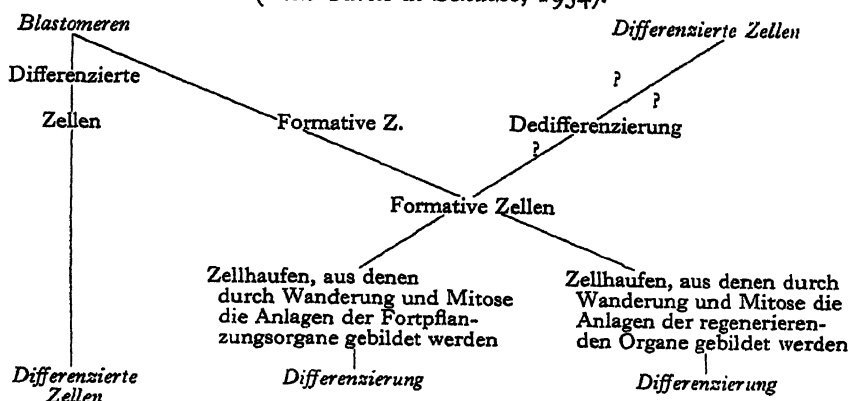


Fig. 5. Parenchym von *Planaria agilis* (a) und *Procotyla fluviatilis* (b) (nach Curtis u. Schulze, 1934). C.s. Cytoplasma des Syncytiums, G. Darmepithel, L.f.c. grosse "formative Zelle", M.f. Muskelfasern, N.s. Kern des Syncytiums.

Tabelle III. Geschichte der formativen Zellen bei den Planarien (nach Curtis u. Schulze, 1934).



Geschlechtliche und ungeschlechtliche Fortpflanzung schliessen sich bis zu einem gewissen Grade aus, was wohl auf dieselbe Materialquelle für beide Vorgänge hindeutet. Vandel meint allerdings, dass daneben auch entdifferenzierte Zellen eine Rolle spielen, dass aus zwei Gewebekomponenten sich das durchgeschnürte Tier ergänzt. Diese letztere Frage bedürfte wohl noch einer Nachprüfung.

Auch bei dem Neuaufbau der Organe etwa nach Reduktion, sollen nach Vandel (1921b) dedifferenzierte Zellen eine sehr grosse Rolle spielen, sodass z. B. dedifferenzierte Zellen aus abgebauten Geschlechtsorganen Material für den Aufbau eines Pharynx liefern können.

Zusammenfassung. Über die Natur des Regenerationsmaterials der Turbellarien bestehen zwei entgegengesetzte Ansichten. Nach der einen Meinung (Lang, Stevens, Steinmann, Hein, Vandel) sind dedifferenzierte Körperzellen, vor allem des Parenchyms, die Quelle der Regeneration. Nach der anderen Ansicht (Bartsch, Curtis, Wilson, Weigand) gibt es besondere "formative" Zellen, die das Regenerat bilden. Anzahl und Verteilung dieser Zellen soll der Grund für die verschiedene Regenerationsfähigkeit der Turbellariengruppen sein. Im Verhalten gegen Bestrahlung lässt sich eine gegen Radiumstrahlen besonders empfindliche Zellart erkennen.

Durch Einwanderung solcher Zellen in das Ektoderm und Entoderm, auch im normalen Tiere, werden die histologischen Bilder der Regenerate sehr unübersichtlich. Wundheilung muss als ein von der Regeneration unabhängiger Vorgang angesehen werden, der vor allen Dingen durch den Reiz des Aussenmediums in Gang gebracht wird.

V. NEMERTINES.

Bei der den Turbellarien im allgemeinen ähnlichen Organisation der Nemertinen kann man erwarten, dass auch die Histogenese der Regenerate in ähnlicher Weise verläuft. Die bedeutende Regenerationsfähigkeit dieser Gruppe ist schon lange Zeit bekannt gewesen.

Erst die Arbeit von Dawydoff (1909) geht näher auf den Anteil der Gewebe beim Aufbau des Regenerates ein und spricht dem Parenchym die Hauptrolle zu, unter Mitwirkung von Ektoderm und Entoderm. Sodann hat Oxner (1909) bei *Lineus* eine entsprechende Art der Regeneration nachgewiesen.

Oxner unterscheidet bei *Lineus ruber* die Formen A und B, von denen A sehr schlecht, B besonders gut regeneriert. A ist später als breite, B als schmale Form von *ruber* bezeichnet worden.

Besonders war aber der Nachweis, dass ein neuer Darm auch in solchen Teilstücken entsteht, die gar kein Entoderm mehr besitzen (Dawydoff, 1910), geeignet, die Frage nach der Herkunft des Regenerationsmaterials aufzurollen. Dawydoff kommt zu dem Schluss, dass Mesodermelemente den neuen Darm bilden, die aus dem Parenchym und den Wandungen der Seitengefässe stammen. Wanderzellen erwähnt er nicht. Auf breiterer Basis nahmen nun Nusbaum u. Oxner (1910a, b, c, 1911a, b, 1913) in einer Reihe von Arbeiten die Untersuchung dieser Frage auf. In der ersten Arbeit, deren Objekt *Lineus ruber* war, führen die Autoren die Regeneration auf Entdifferenzierung und Stoffzufuhr durch Wanderzellen zurück. Diese

Ansicht wird auch in der späteren Arbeit (1910b) vertreten: Es sollen die Wanderzellen in grossen Mengen in die abzubauenen Organe einwandern. In einer weiteren Arbeit (1910c) schildern die Autoren die bei Nemertinen so häufige Cystenbildung, bei der Wanderzellen, Drüsenzellen, Hautepithelzellen und Geschlechtszellen aus dem Gewebe auswandern und gewissermassen für die Verdickung der Hülle verbraucht werden. Es ist also kein Wunder, wenn die Lebenskraft solcher Cysten sehr herabgesetzt ist, da sie überhaupt erst unter ungünstigen Bedingungen auftreten.

Später stellten Nusbaum u. Oxner (1911a) fest, dass die Wanderzellen aufgelockerte Parenchymzellen sind, die den Darm von sich aus aufbauen können. Sie beladen sich zuerst mit Reservestoffen, wandern dann auf den Regenerationsort zu und bauen neues Gewebe auf. In der folgenden Arbeit (Nusbaum u. Oxner, 1911b) wird der Aufbau des neuen Gehirns aus dem Epithel nachgewiesen.

Lässt man *Lineus ruber* hungern (Nusbaum u. Oxner, 1912), so leisten auch dabei die Wanderzellen die grösste Arbeit, denn sie bauen Organteile ab und beladen sich dabei mit grossen Mengen von Nährstoffen. Die degenerierenden Organe verwandeln sich in Syncytien und alle Gewebe nehmen einen mehr embryonalen Charakter an. Die meisten Wanderzellen gehen im Darmgewebe, wohin sie Einschlüsse befördern, zugrunde.

Schliesslich haben die beiden Autoren noch *Lineus lacteus* untersucht (1913). Dabei stellten sie einen doppelten Ursprung der Wanderzellen fest: einesteils aus unpigmentierten Wanderzellen durch direkte Umwandlung, anderenteils aus dem Parenchym, dessen Zellen durch Lockerung frei werden. Auch andere mesodermale Elemente, z. B. die Endothelzellen der Seitengefässe, nehmen an der Umwandlung teil. Die Regenerationsfähigkeit von *L. lacteus* ist gegenüber *L. ruber* polarisiert, während bei *L. ruber* alle Teile des Organismus gleichmässig gut regenerieren.

Dawydoff (1928) verfolgte Reduktionserscheinungen bei den Gattungen *Lineus* und *Cephalothrix*. Nach seiner Meinung können solche Teile bis zu einem Embryonalstadium, ähnlich einer Morula, zurückkehren. Im Inneren der Zellkomplexe sah er embryonale Zellen in mehreren Schichten, die vielleicht den drei Körperschichten entsprechen.

In den letzten Jahren sind aber nun die Regenerationsversuche an Nemertinen von Coe (1929 ff.) in grossem Masstabe durchgeführt worden und diese Untersuchungen haben uns ganz bestimmte Vorstellungen auch über die Herkunft des Materials vermittelt. In der ersten Arbeit (1929) geht der Verfasser von der Tatsache der Autotomie aus. Die Teilstücke regenerieren gleichmässig gut, soweit sie nicht nach Encystierung in einer Schleimhülle zugrunde gehen. Für die Versuche wurden die besonders geeigneten Arten *Lineus vegetus* und *L. socialis* verwendet, daneben—mit geringerer Regenerationsfähigkeit—*L. pictifrons*, *L. flavescens* und *L. ruber*.

Bei der Wundheilung wird nach anfänglicher Kontraktion der gesamten Muskulatur viel Schleim ausgeschieden, der die Wunde bedeckt. Für den Aufbau des Regenerates kommen nach dem Autor drei Zellsorten in Frage: (1) phagocytierende Wanderzellen aus dem Parenchym, die das zerfallende Gewebe auf-

nehmen; (2) formative Zellen mesodermalen Ursprungs, die hinzuwandern und Muskulatur, Bindegewebe und Parenchym im Regenerat bilden; (3) die Neoblasten, die für dedifferenzierte Epithelzellen angesehen werden (Basalzellen des Integumentes) und den wesentlichen Teil der Regenerationsknospe ausmachen. In der Cyste kann es zu Rückbildungen kommen, wobei das Gewebe auf einem niederen Differenzierungszustande verharren kann.

In der zweiten Arbeit schildert Coe (1930a) das Resultat der Regeneration längsgeschnittener Körperteile, besonders des Kopfabschnittes. Dabei ist zunächst die Wunde durch ein dünnes Epithel überdeckt worden, dessen Zellen aus den tieferen Epithellagen und aus dem Mesenchym (Neoblasten) stammen. Aus solchen Epithelzellen soll auch die neue Hirnhälfte hervorgehen, an anderer Stelle der Arbeit (Zusammenfassung) wird allerdings der Ursprung der Gehirnhälften auf Neoblasten zurückgeführt. Die bestehende Gehirnhälfte wird durch Phagocyten abgebaut, bis sie in der Grösse der neuentstehenden entspricht. Die übrigen Gewebe des Kopfstückes werden nach dem Autor im wesentlichen von Neoblasten gebildet (in der Zusammenfassung handschriftlich korrigiert!): So die beiden Lateralnerven, deren Anwesenheit Coe für das Zustandekommen des Regenerates notwendig erachtet, denn Teilstücke ohne ein Stück dieser Längsnerven regenerieren nie. Die gleiche Herkunft war für die Muskulatur festzustellen.

In einer späteren Arbeit berichtet Coe (1932) über die Regeneration von *L. pictifrons*. Hier weist er daraufhin, dass das Reorganisationszentrum die Region der cerebralen Sinnesorgane und kurz dahinter ist: Kopfstücke, denen diese Region fehlt, regenerieren nie. Das Material für diese Vorgänge sieht der Autor ebenfalls in Mesenchymzellen (handschriftlich korrigiert).

In einer weiteren Untersuchung hat Coe (1934a) die Erforschung der Materialherkunft im wesentlichen zum Abschluss gebracht. Er erklärt, dass bei der Regeneration der Heteronemertinen die Anwesenheit eines Stückes der Längsnerven notwendig ist. Die Wunde wird durch Epithelzellen bedeckt, die aus basalen Lagen der Epidermis stammen. Im übrigen werden alle Organe von Neoblasten (Mesenchymzellen, Regenerationszellen) aufgebaut, mit Ausnahme der Epidermis der cerebralen Sinnesorgane, des Mundes und der Begrenzung des Vorderdarmes, die von epidermalen Zellen abstammen.

Die Regenerationsfähigkeit der einzelnen Körperabschnitte bei fünf untersuchten *Lineus*-arten gibt Fig. 6 wieder. Damit vergleicht Coe die Regenerationsfähigkeit bei anderen Nemertinegattungen, speziell den Hoplonemertinen, die besonders Vorderstücke nur geringfügig regenerieren.

Für die regenerativen Prozesse muss eine Polarität der zuwandernden Zellen angenommen werden, nur die epithelialen Zellen sind davon frei. Der Wundheilungsprozess ist keine Dedifferenzierung von Epithelzellen, sondern entspricht dem Nachschub von Zellen aus den basalen Lagen der Epidermis. Darunter sammeln sich dann in einem Flüssigkeitsraum die mesenchymalen Zellen, die Reservezellen aus dem gesamten Körperbindegewebe sind. Die Unterscheidung zwischen Mesenchym und Neoblasten, die Coe früher aufrecht erhielt, erklärt er jetzt als hinfällig. Schliesslich treten noch Wanderzellen aus dem Parenchym zum

Abbau der Gewebereste auf. Aus dem Blastem unter dem Epithel geht selbständig das Gehirn und der im regenerierenden Kopfteil überhaupt nicht vorhandene Darmabschnitt hervor. Rüssel, Rüsselscheiden und Mitteldarm stammen ebenso aus mesenchymalem Material.

Coe konnte auch feststellen, dass, wenn bei längsgespaltenen Stücken von *L. socialis* und *L. vegetus* das Epithel die Wunde überdeckt hat und diese wieder geöffnet wird, eine grössere Anzahl Köpfe oder Schwanzstücke gebildet werden,

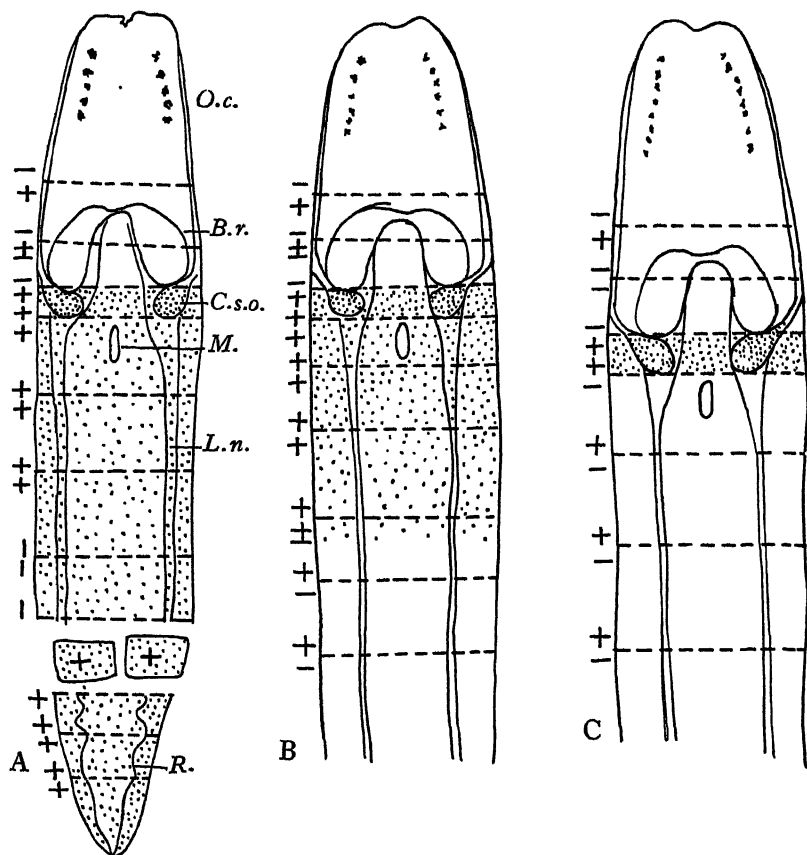


Fig. 6. Schemata zur Demonstration der Regenerationsfähigkeit (nach Coe, 1934). A, *Lineus socialis* und *L. vegetus*; B, *L. pictifrons* und *L. rubescens*; C, *L. ruber*. B.r. Gehirn, C.s.o. cerebrale Sinnesorgane, L.n. Lateralnerv, M. Mund, O.c. Augen, R. Rektum. Punktierte Regionen liefern Vorder- und Hinterregenerate, + = vollständige Regeneration und Regulation, ± = gelegentliche Regeneration, - = unvollständige Regeneration.

was wohl als Ausdruck für eine ungehemmte Tätigkeit der Mesenchymzellen, die nunmehr von dem überdeckenden Epithel befreit sind, anzusehen ist.

Coe nimmt an, dass der überall notwendige Nervenstrang die Regenerationszellen aktiviert und das Regenerationszentrum bei den meisten Formen direkt hinter dem Gehirn liegt, bei manchen Formen aber sich bis zum Vorderdarm erstreckt, bei zwei Arten allerdings sogar bis zum Hinterende reicht.

Coe (1934a) fasst die Resultate seiner Untersuchungen zusammen und stellt fest, dass Regeneration am Hinterende mehr nur eine andere Form des Wachstums ist, insofern als die zuwandernden Regenerationszellen in die vorhandenen Gewebe aufgenommen werden. Anders bei der Regeneration des Vorderendes (Fig. 7): Hier wird zunächst ein undifferenziertes Blastem gebildet, aus dem gewisse Organe sich selbständig herausdifferenzieren, ohne Anschluss an die alten Gewebe. Der Autor kann aber nicht entscheiden, ob etwa einige dieser Bildungszellen aus der Epidermis stammen, die das Mesenchym bedeckt, eine Vermutung, die recht unwahrscheinlich ist. Auch diese müssten dann allerdings als totipotent angesehen werden. Für die Bildungszellen des neuen Gehirns gilt, dass sie in enger topo-

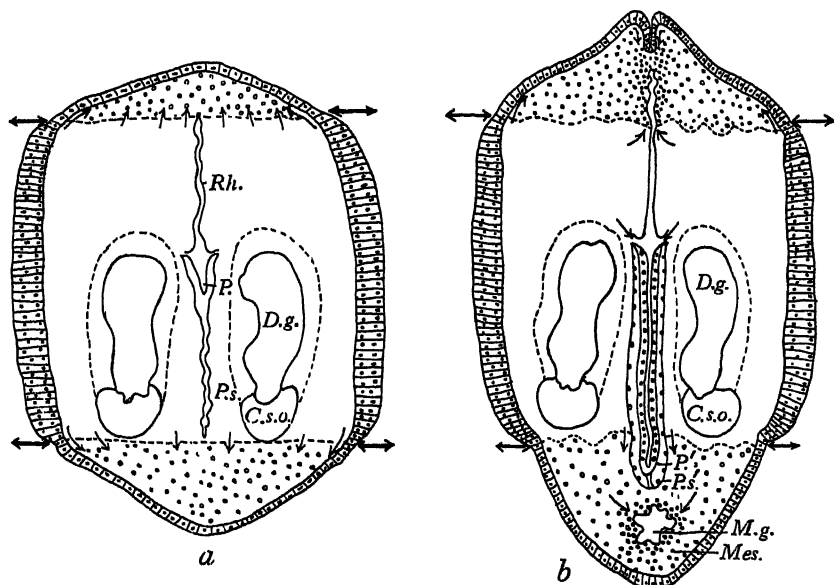


Fig. 7. *Linus socialis*. Frühes (a) und späteres (b) Regenerationsstadium einer Kopfpartie, die die cerebralen Sinnesorgane (C.s.o.) enthält (nach Coe, 1934). D.g. Gehirn, Mes. Mesenchym, M.g. Anlage des Mitteldarmes, P. Rüssel, P.s. Rüsselscheide, Rh. Rhynchodäum. Die Pfeile zeigen die Wanderrichtung der Zellen.

graphischer Beziehung zu der Epidermis stehen. Vielleicht sind sie also determiniert als Ektodermabkömmlinge.

Die verschiedene Regenerationsfähigkeit der einzelnen Formen führt Coe auf die verschiedene Ausdehnung der Regionen, die Regenerationszellen (Neoblasten) enthalten, zurück. Also liegen ganz ähnliche Verhältnisse vor, wie bei den Turbellarien. Vielleicht behalten die Mesenchymzellen bei den schlecht regenerierenden Formen auch mehr den Charakter von Bindegewebezellen, als bei den Formen mit guter Regeneration.

Neben den hauptsächlich untersuchten Heteronemertinen liegt auch eine Untersuchung über die Hoplonemertine *Prostoma* von Kipke (1932) vor. Bei *Prostoma graecense* wurde nach dem Eingriff die Wunde ebenfalls durch eine dünne Epithelmembran geschlossen, deren Zellen aus dem alten Epithel stammen.

Bei sehr grossen Wunden traten auch die Regenerationszellen dazu. Die Wiederherstellung der meisten Organe ist von der Anwesenheit des Rüssels abhängig. In manchen Fällen konnte die Neubildung des Gehirns unterbleiben. Es scheint also zweifelhaft, ob bei diesen Versuchen das Nervensystem von gleich entscheidender Bedeutung ist, wie es Coe für die *Lineus*-regeneration annimmt. Das Regenerationsvermögen ist verglichen mit dem der *Lineus*-arten etwa von mittlerer Stärke.

Zusammenfassung. Das Baumaterial der Nemertinenregenerate ist das Mesenchym, von Coe als Neoblasten bezeichnet. Dagegen glaubten Nusbaum u. Oxner, dass in den Wanderzellen, wie sie die freien Zellen nannten, dedifferenziertes Parenchym vorläge. Die Ektodermzellen sollen sowohl bei der Wundheilung entscheidend tätig sein, als auch werden nach den älteren Arbeiten alle ektodermalen Bildungen aus ihnen abgeleitet. In einer seiner letzten Arbeiten dagegen spricht Coe von den Epithelzellen tieferer Lagen, die die Regeneration ermöglichen sollen. Wahrscheinlich sind das sog. Basalzellen, die relativ undifferenziert sind.

Bei Reduktionsvorgängen geht die Dedifferenzierung bis zu totipotenten Zellen zurück (Dawydoff, 1928), aus denen dann der neue Organismus sich bildet.

VI. POLYCHAETA.

In den Untersuchungen über die Regeneration der Polychäten ist schon frühzeitig die Frage nach der Herkunft des Materials aufgetaucht. In erster Linie wird in den älteren Arbeiten die Neubildung des Darmes diskutiert, eine Frage die zahlreiche Schwierigkeiten bot, da die Grenze zwischen Ektoderm und Entoderm am Polychätenkörper nicht immer leicht zu bestimmen ist. Dahinter stand aber die prinzipielle Frage, ob bei Regeneration die Organe von denselben Keimblättern wiederhergestellt werden, die sie in der normalen Entwicklung bilden. Damit wurde in gleicher Weise Ektoderm und Mesoderm in den Bereich der Prüfung gezogen. Wenn man die Resultate der älteren Untersuchungen zusammenfassen will, so kann man sagen, dass nach den Ansichten von Michel (1898), E. Schultz (1899), J. Nusbaum (1905, 1908) und Iwanoff (1906–7, 1908) das Entoderm aus dem alten Entoderm, alles Übrige aus dem Ektoderm gebildet wird. Auf diesem Standpunkte steht auch Langhammer (1928). Allerdings konnten Nusbaum und Iwanoff schon eine Beteiligung des Mesoderms nachweisen.

Iwanoff (1928) hat in seinen Betrachtungen über das Verhältnis von Ontogenese und Regeneration die Schwierigkeiten aufgedeckt, die einer histogenetischen Betrachtung der Regenerationsvorgänge im Wege stehen. Er stellte fest, dass nicht nur das Ektomesoderm, sondern auch das cölomatische Mesoderm aus dem Ektoderm am auswachsenden Hinterende wie im Regenerat hervorgeht. Wie aus seinen Bildern zu entnehmen ist, besteht ein histologischer sichtbarer Unterschied zwischen Ektoderm und Mesoderm nicht: in beiden Schichten sehen die Zellen und ihre Kerne gleich aus. Daraus ergibt sich aber eine grosse Schwierigkeit der Beurteilung, ob Ektodermzellen ins Mesoderm wandern und dieses bilden oder ob das Ektoderm aus der darunterliegenden Mesodermlage aufgebaut wird. Ja, am Hinterende des Polychätenkörpers ist die Unterscheidung der beiden Körper-

schichten noch nicht eigentlich zulässig, wie die Bilder deutlich lehren. Iwanoff meint auch, dass nicht Ektodermzellen in das Mesoderm überwandern, sondern dedifferenzierte Zellen. Das Vorhandensein von grossen Zellen im Mesoderm bei gewissen Polychäten führt Iwanoff zu der Ansicht, dass auch das alte Mesoderm sich beim Aufbau des neuen beteiligt.

Eine neue Untersuchungsperiode wird durch die Arbeit Pflugfelders (1929) eingeleitet. Er untersuchte die Regeneration an einem tropischen Vertreter, *Diopatra amboinensis*. Hier besteht eine enorme Regenerationsfähigkeit. Auch Pflugfelder sah das ekto- und entodermale Epithel aus den entsprechenden alten Epithelien hervorgehen. Aber bei dem Aufbau des Mesoderms wurde die Zuwanderung von sog. Regenerationszellen beobachtet. Pflugfelder schildert diese Zellen als spindelförmig. Sie liegen gewöhnlich im Bindegewebe in der Nähe der Blutgefässe und der Muskulatur. Vor allem aber umgeben sie die Kolossalfasern des Bauchmarks. Nach der Wundsetzung verändern sie ihre Färbbarkeit, lösen sich aus dem Bindegewebe und wandern in ganzen Zügen auf die Wundstelle zu entlang der Längsmuskulatur und vor allem längs des Bauchmarks. Pflugfelder macht aber auch darauf aufmerksam, dass das sich über die Wunde legende Epithel nicht nur die Form, sondern auch die Färbbarkeit verändert und einen embryonalisierten Eindruck macht; ähnliches beobachtet er beim Darmepithel, wenn hier die Dedifferenzierung auch nicht so weitreichend war.

Für die Sylliden kam Okada (1929) zu dem Ergebnis, dass alle mesodermalen Gewebe eine Entdifferenzierung zu Mesenchym durchmachen und aus diesem Mesenchym das Material der mesodermalen Gewebe des Regenerates stammt. Auch wurde eine Dedifferenzierung am Wundrande von diesem Autor nachgewiesen.

Das Vorkommen undifferenzierter Zellen im ungestörten Polychätengewebe ist nun auch weiterhin von verschiedenen Autoren bestätigt worden. Zu gleicher Zeit mit den soeben genannten Arbeiten zeigte Stolte (1929) bei *Polyophthalmus pictus* das Fehlen jeglicher Regenerationsvorgänge auf. Nur Wundheilung tritt hier am Vorder- und Hinterende ein. Das Material dafür stammt aber aus dem Mesoderm und Stolte konnte solche indifferenten Zellen in Ansammlungen unter der Epidermis liegen sehen.

Den ganzen Fragenkomplex hat in den letzten Jahren Probst nach Untersuchungen an verschiedenen Polychäten behandelt. In einer vorläufigen Mitteilung (1930) teilt er mit, dass bei der zu Autotomie neigenden *Aricia foetida* Regenerationszellen über dem Bauchmark und an der Hinterseite der Dissepimente liegen. Es scheint, dass alle Gewebe im Regenerat davon ihren Ursprung nehmen.

In der ausführlichen Arbeit schildert Probst (1931) näher die Verteilung der Regenerationszellen im unverletzten Tier. Sie besitzen im Ruhezustand kleine unregelmässig geformte Kerne, die sich tiefdunkel färben. Zellgrenzen sind nicht nachzuweisen, sondern das Ganze wirkt wie ein Syncytium (Fig. 8a). Zu Beginn der Regeneration vergrössern sich die Kerne, oft schon eine halbe Stunde nach dem Eingriff. Ihr Inhalt wird deutlicher und eine Kernmembran ist sichtbar. Das Plasma, das vorher fädig war, ist jetzt homogener geworden (Fig. 8b). Später vergrössern sich die Kerne noch weiter, vor allem bekommen sie einen sehr grossen

Nukleolus. Sie ahneln jetzt den Neoblasten der Oligochäten (s. u.). Zellgrenzen im Plasma sind nicht zu erkennen. Damit haben die Regenerationszellen wohl

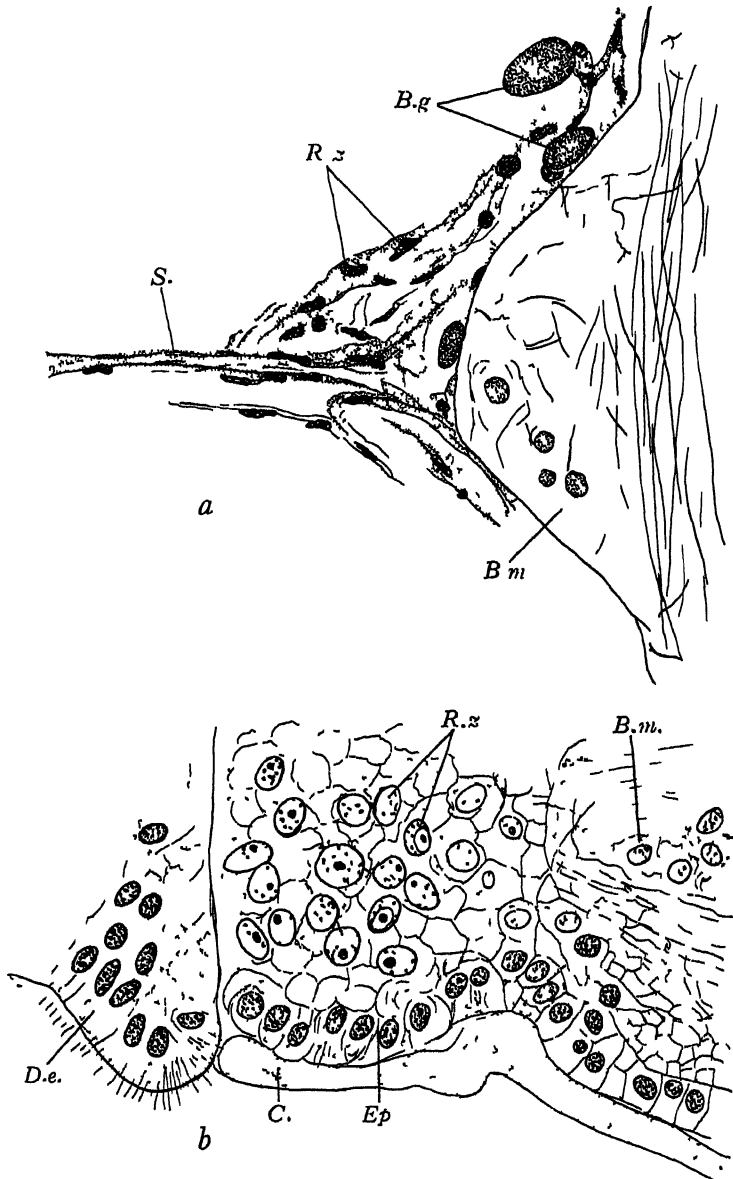


Fig. 8. *Arcia foetida* a, Bauchmark und Dissepiment eines unverletzten Tieres b, Ansammlung von Regenerationszellen am 4. Tage der Regeneration am Ende des Bauchmarkstumpfes, 666 \times (nach Probst, 1931). B.g. Blutgefasse, B.m. Bauchmark, C. Cuticula, D.e. Darmepithel, Ep. Epidermis, R.z. Regenerationszellen, S. Septum.

ihr Reifestadium erreicht. Diese Zellen wandern nun nach dem Eingriff in zwei geschlossenen Strömen rechts und links vom Bauchmark an die Wunde heran.

Die Aktivierung der Regenerationszellen greift bei *Aricia* auf 10 Segmente über, ob aber alle diese Zellen schon vorher bereit lagen, oder ob etwa Peritonealzellen im weiteren Verlauf der Einwirkung der Wunde aktiviert werden, war nicht zu entscheiden. Der Zustrom von Regenerationszellen an der Wundstelle führt schliesslich dazu, dass die alten Wundränder auseinandergedrängt werden und so ein Blastem entsteht. Das geschieht bei *Aricia* etwa am 5. Tage. Nun wird an der Oberfläche dieses Blastems ein Epithel gebildet. Nach dem 4. Tage treten in dem Blastem aber auch die ersten Mitosen auf, die vorher völlig fehlten. Man geht wohl nicht fehl, wenn man von diesem Zeitpunkt den Beginn der Differenzierung rechnet. Am 6. Tage ist das Epithel bereits durch eine Basalmembran von dem darunterliegenden mesodermalen Gewebe abgeteilt. Aber Zellgrenzen sind in diesen beiden Gewebeschichten noch nicht festzustellen. Damit sind die ersten Vorgänge der Regeneration, die allein Aufschluss über die Herkunft des Materials geben können, abgeschlossen. Von älteren Angaben dieser Art ist hier nur die von Czerski u. Nusbaum (1905) zu nennen, die ebenfalls schon diese Zellströme rechts und links des Bauchmarks beobachteten.

Probst konnte aber auch zeigen, dass durch die Wundsetzung eine Entdifferenzierung der Epidermis zunächst nicht erfolgt. Erst wenn Regenerationszellen in ihrer Nachbarschaft liegen, kommt es zu Aufquellung des Gewebes und der Kerne. Dagegen degeneriert weitgehend die Ringmuskulatur, die alten Nervenzellen und die Längsmuskeln bleiben von dem Regenerationsgeschehen unberührt, wenn auch die alten Nervenfasern in die neuen Teile einwachsen. Für den Darm war nicht zu entscheiden, ob Regenerationszellen ihn innerhalb des Regenerates aufbauen oder ob das alte Darmepithel eine Umwandlung und Neubildung aus eigenen Kräften durchmacht. Schliesslich sind auch Quellungserscheinungen nach Wundsetzung an den Septen festzustellen. Ob Septalzellen auswandern, ist ungewiss.

Diese Resultate hat Probst (1932) an einem anderen Polychät, *Owenia fusiformis*, nachgeprüft. Diese Form unterscheidet sich im Regenerationsgeschehen wesentlich von der vorher genannten *Aricia*. Beim Wundverschluss kommt es erst spät zum Zusammenschluss der ekto- und der entodermalen Epithellage und im Anschluss daran legen sich die Zellen des benachbarten Hautepithels über die Wunde. Im einzelnen geht diese Umwandlung so vor sich, dass Basalmembran und plasmatische Strukturen verschwinden und die Kerne aufgelockerter erscheinen. Sie stehen auch weiter auseinander als vorher. Von unten her treten offenbar allerlei mesodermale Elemente ein, denn man kann später Muskeltrümmer u. a. in der Epidermis nachweisen. Dadurch lässt sich eine Lockerung des epidermalen Gewebes erkennen, die einer Entdifferenzierung wohl gleich kommt. Jetzt erst treten Mitosen auf (nach 4 Tagen), und später sind an der Basis des Epithels wieder die sog. Basalzellen sichtbar. Ganz entsprechend verhält sich bei *Owenia* das Darmepithel.

Die Regeneration des Mesoderms verläuft in besonderer Weise: Nach dem Eingriff erfolgt eine Zertrümmerung vor allem der Muskulatur. Diese Fragmente gelangen in die Leibeshöhle, werden dort weiter abgebaut und treten schliesslich als mesenchymatisierte Zellen in das Regenerationsblastem, das sich zur neuen

Mesodermanlage entwickelt und Cölomblasen abschnürt. Probst macht schliesslich noch darauf aufmerksam, dass ähnlich wie bei der Regeneration auch bei der Gonadenbildung ein Abbau sämtlicher mesodermaler Strukturen, insbesondere der Muskulatur, eintritt.

Bezüglich der Materialbeschaffung bei der Regeneration dieser beiden Arten bestehen also wesentliche Unterschiede. Gemeinsam ist beiden aktive Regeneration, Umwandlung der an die Wunde angrenzenden Gewebe im Sinne einer Mesenchymatisierung, sowie spätes Auftreten von Mitosen in den Geweben. Bei der Regeneration geht der Anstoss zur Ausbildung eines Blastems im einen Falle (*Aricia*) von Regenerationszellen, im anderen Falle (*Owenia*) von dedifferenzierten Geweben aus.

Diese Angaben finden ihre Bestätigung in Untersuchungen, die Faulkner (1930, 1932) durchgeführt hat. In der ersten Arbeit kommt sie zu dem Resultat, dass bei *Filograna*, die mit der Gattung *Salmacina* identisch ist, Neoblasten existieren, die in zwei medianen Reihen im ventralen Teil der Körperwand angeordnet sind. Diese Zellen bilden Ersatzzellen, Phagocyten, Histoblasten und Gonocyten. Sie haben relativ grosse Kerne, deren Chromatin in Schollen verteilt ist. In dem Ruhestadium fehlen Nukleolen. Diese Zellen ähneln sich auch in ihren ersten Differenzierungsstadien und können alle Gewebearten bilden. Ihre Aufgabe ist also die Funktion ihrer Lage. Bei der Differenzierung entwickeln sich in den Neoblasten kleine Nukleolen. Einem der drei Keimblätter kann man diese Zellen nach Faulkner wohl kaum zuteilen. Denn in der Knospungszone liegen sie meist an der Grenze von Ektoblast und Entoblast, im Hinterende des Wurmes. Sie dienen dem Abbau, zur Neubildung der Gewebe bei Regeneration, bei der Knospenbildung zur Verlängerung der Organe in die Knospe, bei der Gonadenbildung liefern sie die Keimzellen.

In der zweiten Arbeit (1932) beschäftigt sich Faulkner mit der Histogenese nach Entfernung des Hinterendes bei *Chaetopterus variopedatus*. Auch hier fand man auf der Ventralseite der Cölomwände nach dem Körperende zu in immer zunehmender Menge Zellen, die als Neoblasten bezeichnet werden müssen. Sie liegen in einer Reihe zusammengeschlossen über und zwischen den beiden Teilen des Bauchmarks. Aber auch in allen anderen Teilen der Leibeshöhle, vom Darm bis zur Epidermis und den Dissepimenten findet man Neoblasten, besonders in den jüngsten Segmenten.

Die Neoblasten bilden also im normalen Wachstum nicht nur die mesodermalen Organe, sondern nehmen auch an dem Aufbau der ektodermalen und entodermalen Organe teil. Die Anlage der Gonaden geht ebenfalls auf sie zurück. Im ersten Stadium der Wundheilung ist eine Aktivierung der in der Nähe der Wunde liegenden Neoblasten zu bemerken, besonders derjenigen, die zwischen den beiden Hälften des Bauchmarks liegen. Sie bilden ein Mesenchym, das den ganzen Raum zwischen Epithel und Darm ausfüllt. Hier wird auch ein neues Epithel gebildet, das sich mit dem Darmepithel verbindet und eine Regenerationspapille angelegt. In ihr bildet sich ein neues Neoblastenzentrum, das das alte durch die Entfernung des Hinterendes beseitigt ersetzt. Nach Abschluss dieser Vorgänge, gewöhnlich nach drei

Tagen, nimmt die Zahl der Neoblasten am Hinterende wieder ab. Sie liegen aber noch in einem Ring um das Ende des Darmes und der Epidermis. Dieser Zustand dauert etwa bis zum 10. Tage. Dann verteilen sich die Neoblasten allmählich wieder auf ihre Ruheplätze, an den Wänden der neuen Cölomsäcke. Die an der Vorderwand der Dissepimente verteilten Neoblasten geben den Gonaden den Ursprung. Der Unterschied in der Verteilung der Neoblasten in Wachstum und Regeneration besteht als in ihrer Beschränkung auf die interneurale Region im ersteren Falle, in ihrer Ausdehnung über das ganze Wundregion zwischen Ektoderm und Entoderm im zweiten Falle. Alle weitere Neubildung geht von der Regenerationsspille aus.

Zum Schluss sollen hier noch einige Beobachtungen über die Beziehungen totipotenter Zellen zu geschlechtlicher und ungeschlechtlicher Vermehrungsweise besprochen werden: So hat Malaquin (1925) als Abschluss einer Reihe von Mitteilungen über *Salmacina dysteri* mitgeteilt, dass das Material für die Knospungsprozesse den Geschlechtszellen homolog ist und dass beide Arten der Fortpflanzungszellen, die geschlechtlichen und die ungeschlechtlichen, an denselben Punkten im Organismus entstehen. Aber während die Gonocyten zu einer traubigen Gonade auswachsen, wandern die Histoblasten (Blastocyten) in die Gewebe. So wird Epithel, Nervensystem und Muskulatur aufgebaut. Malaquin behauptet, dass das alte Material zugrunde ginge. Über die Bildung des neuen Darmes wird nichts gesagt.

Dehorne (1932) schildert ebenfalls acidophile Reservezellen, die bei den komplizierten Fortpflanzungsvorgängen bei der Gattung *Dodecaceria* eine Rolle spielen.

Zusammenfassung. Im Gegensatz zu der älteren Ansicht, dass das Regenerationsmaterial, mit Ausnahme des Bildungsmaterials für den Darm, aus dem Ektoderm stammt, haben neuere Arbeiten (Pflugfelder, Probst, Faulkner) den Beweis erbracht, dass undifferenzierte Zellen (Neoblasten) wesentlichen Anteil an der Regeneration haben. Allerdings regenerieren die einzelnen Arten sehr verschieden. Die Reihe abnehmender Regenerationsfähigkeit lautet: *Diopatra*—*Aricia*—*Owenia*—*Polyophthalmus*. Bei der letzten Art findet sich lediglich Wundheilung. Auch die Geschlechtszellen konnten in einigen Fällen auf Neoblasten zurückgeführt werden.

VII. OLIGOCHAETA.

Früher als bei allen bisher besprochenen Gruppen ist bei den Oligochäten das Zellmaterial erkannt worden, das den regenerativen Vorgängen zugrunde liegt: Randolph (1892) konnte bei der Regeneration des Hinterendes von *Lumbriculus* grosse Zellen konstatieren, die zwischen Ektoderm und Entoderm liegen und die die Verfasserin Neoblasten nannte. Diese grossen Ausgangszellen verkleinern sich im Verlaufe mitotischer Vorgänge und regenerieren das sich neu bildende Hinterende. Bei einem Vergleich mit anderen Oligochätengruppen kommt Randolph zu dem Schlusse, dass Regenerationsfähigkeit vom Vorhandensein von Neoblasten abhängig ist.

Bestätigungen dieser Ansicht findet man bei Iwanoff (1903), Dalla Fior (1908), Lomb (1910) und bei Kreckler (1910), die als Material *Lumbriculus* bzw. *Tubifex*, *Stylaria* und *Limnodrilus* verwendeten. Zur Bildung des Mesoderms dienen also nicht ubertretende Ektodermzellen, sondern Neoblasten. Dagegen soll nach Kreckler die Ringmuskulatur aus dem Ektoderm regeneriert werden. Das Entoderm regeneriert sich immer aus dem Stumpf. Wenn aber bei Ausfall des Entoderms die Regeneration des Hinterendes unterbleibt, sammeln sich die Neoblasten am Hinterende zu einem Zellhaufen an. In dem Ektoderm der Regenerationszone nehmen die Zellen das Aussehen von Neoblasten an, von denen sie unterlagert werden. Es müssen also wohl Einflüsse von den Neoblasten ausgehen, die auf die Ektodermzellen wirken. Kreckler konnte auch Wanderung der Neoblasten nach vorn feststellen, nur in die ersten 10 Segmente treten sie nicht ein. L. Dehorne (1916-18) sieht die Neoblasten irrtümlicherweise als Muskelzellen an.

In einer weiteren Arbeit beschäftigt sich Kreckler (1923) ausführlich mit Herkunft und Tätigkeit der Neoblasten. Kreckler untersuchte in dieser Arbeit hauptsächlich *Limnodrilus* und *Tubifex*. Während die älteren Autoren diese Zellen hauptsächlich längs des Bauchmarks verteilt sahen (Chordazellen Sempers), sah sie Kreckler an der Hinterwand der Septen, längs der Blutgefässe und zwischen Darm und Chloragogen. In dieser neueren Untersuchung schien ihm zweifelhaft, ob er früher nicht Phagocyten für solche Neoblasten angesehen hatte. Die letzteren lassen sich am besten daran erkennen, dass sie in Nestern auftreten. Von den Phagocyten unterscheiden sich die Neoblasten in ihrem Plasma nur wenig, dagegen sind Kern und Nukleolus verhältnismässig gross. Zellteilung tritt bei den inaktiven Phagocyten auf, bei den Neoblasten dagegen erst an der Stelle, wo sie in Aktion treten. Bei der Aktivierung werden Kern und Nukleolus der Neoblasten immer grösser, während bei den Phagocyten hauptsächlich das Cytoplasma durch Vakuolenbildung verändert wird (Fig. 9). Diese Unterscheidung liess nun eine genauere Bestimmung der Verteilung der Neoblasten zu: Sie finden sich überwiegend an der Hinterseite der Septen und zwar ventral in dem Winkel zwischen dem Septum und der Körperwand, wenigstens im normalen Tiere: Bei Tieren, an denen Eingriffe vorgenommen wurden, ist die Verbreitung der Neoblasten eine weiter ausgedehnte. An den Septen liegen ruhende kleinere und aktivierte grössere Neoblasten. Sie müssen als Reste undifferenzierter Zellen angesehen werden, die bei der Bildung der Colomblocks in der Embryogenese wie nach Regeneration dort liegen geblieben sind.

Am Septum sind die Frühstadien der Neoblasten am häufigsten dorsal zu finden, die Reifestadien dagegen liegen meistens ventral. Erst wenn die Zellen ihre definitive Grösse erreicht haben, verlassen sie das Septum. Sie wandern dann längs des Nervensystems. Der erste Effekt der Wundsetzung ist also wohl eine physiologische Veränderung der Neoblasten, die in der Grössenzunahme hauptsächlich des Kernes zum Ausdruck kommt. Ein weiterer Stimulus veranlasst dann die Wanderung auf die Wunde zu. Die Wanderung nach vorn scheint bei *Tubifex* und *Limnodrilus* beschränkt zu sein, was in einer mangelhaften Regeneration an vorderen Wundflächen zum Ausdruck kommt. Die Wirkung der Wundsetzung als stimulierender

Faktor reicht im ganzen etwa 7 Segmente weit, eine stärkere Mobilisation der Neoblasten erfolgt aber nur in den benachbarten vier Segmenten. Dieser Reiz veranlasst also nicht nur die Wanderung dieser Zellen, sondern auch die Umdifferenzierung der noch ruhenden Neoblasten. Ihre Aktivierung ist

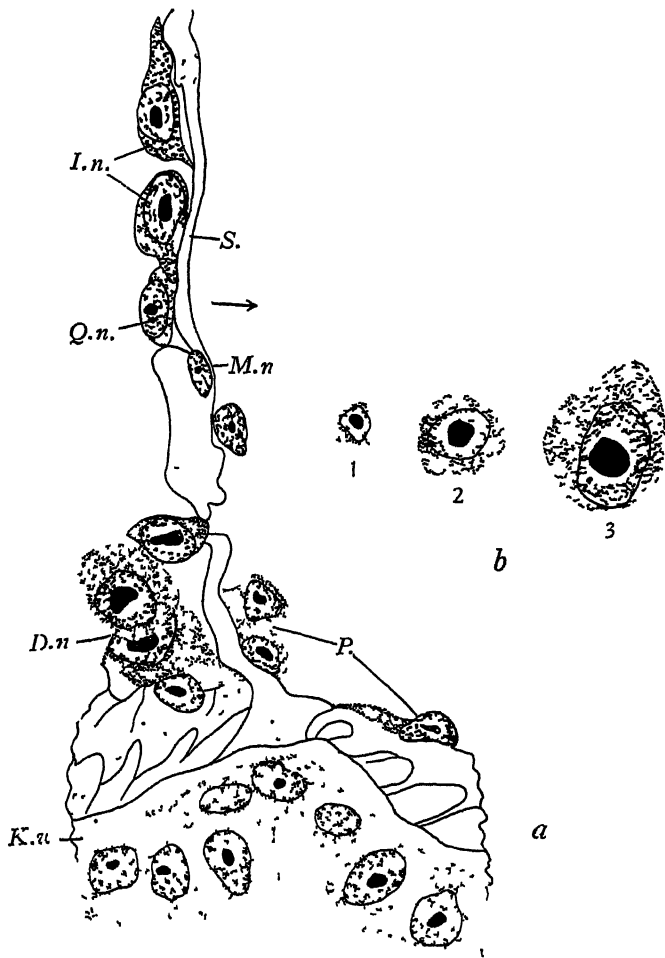


Fig. 9. *Lymnodrilus spec.* a, Metamorphose der Neoblasten am Dissepiment. b, Anfangs- (1), Übergangs- (2) und Endstadium (3) der Neoblastenmetamorphose (nach Kreckler, 1923). D.n. definitive Neoblasten, I.n. Übergangsstadium, K.w. Körperwand, M.n. Muskelzellkerne, P. Phagocyten, Q.n. Ruhende Neoblasten, S. Dissepiment. Der Pfeil zeigt nach vorn.

wahrscheinlich erst dann abgeschlossen, wenn die Bildung neuer Segmente mit Neoblastennestern beginnt. Der weitere Regenerationsvorgang entspricht dem Wachstum völlig. Die erste Proliferationsphase der Regeneration wird durch die Neoblasten bestimmt, die zweite, die formative Phase, dagegen von dem Darm, der die Bildung neuer Segmente beeinflusst. Nuzum u. Rand (1924) haben behauptet, dass gegebenenfalls das Pharynxepithel in der Lage sei Material zur Regeneration

des Gehirns zu liefern. Daneben liefert aber auch das alte Bauchmark Material. Diese aus dem Pharynx stammenden Zellen haben Kerne wie die Neuroblasten. Es ist zweifelhaft, ob sich diese Beobachtung bestätigen wird und es scheint mir wahrscheinlich, dass es sich bei diesen Zellen um Peritonealzellen handelt.

Die nächste Etappe in der Untersuchung der Oligochäten bildet die Arbeit von Hämmerling (1924) an *Aeolosoma*. Hier wird die Frage untersucht, ob Entdifferenzierung oder Differenzierung undifferenzierter Zellen bei der ungeschlechtlichen Fortpflanzung und Regeneration vorliegt. Hämmerling kommt für sein Untersuchungsobjekt *Ae. hemprichi* zu der Auffassung, dass das Epithel sowohl bei der Knospung wie bei Regeneration allein aus den Neoblasten aufgebaut wird. Auch für die Neubildung des Darmes nimmt Hämmerling das Gleiche an.

In der Arbeit findet sich übrigens auch eine Zusammenstellung über die Angaben aller bisherigen Untersucher bezüglich des Vorkommens der Neoblasten. Die meisten Autoren lassen das Mesoderm aus Ektoderm hervorgehen, oder aus altem Mesoderm. Nur die bisher von mir genannten Autoren sprechen den Neoblasten diese Aufgaben zu. Hämmerlings Zusammenstellungen in seinen Tabellen I und II entheben mich des weiteren Eingehens auf diese Frage. Hämmerling führt also Epidermis, Darm, Bindegewebe, Teile der Nephridien und den Pharynx direkt auf die Neoblasten zurück, während Borstensäcke, Ringmuskulatur und Gehirn durch Umdifferenzierung aus der Epidermis entstehen sollen.

Im Jahre 1927 konnte Stolte nachweisen, dass im individuellen Leben alle Gewebe der Naiden Nachschub von Zellen erhalten, die er Blastocyten nennt. Der Altersprozess tritt dort ein, wo dieser Nachschub ausbleibt, z. B. im Darm-Chloragogensystem. Es ist allerdings in dieser Arbeit noch nicht entschieden, ob der Mangel an Blastocyten oder an Phagocyten dieses Verdauungssystem zusammenbrechen lässt.

Eine Einwanderung von Blastocyten liess sich dagegen in der Teilungszone in alle Gewebe verfolgen, selbst in den Darm. Weitzmann (1927), der die Mesoderm-elemente untersuchte und ihren Anteil am Regenerat vergleichend bei Limikolen und Terrikolen (*Lumbriculus*, *Rhynchelmis*, *Eisenia*) feststellte, konnte für *Lumbriculus* nachweisen, dass Ruhestadien, die noch nicht die eigentliche Grösse von Neoblasten haben, sehr häufig sind. Ihre Mobilisierung erfolgt mit Beginn der Regeneration oder der Wundsetzung. Weitzmann nimmt also für *Lumbriculus* an, dass die primäre Gestalt der Blastocyten die gewöhnlicher Peritonealzellen ist.

Ganz anderer Herkunft dagegen sind die Regenerationszellen bei den Lumbriciden (*Eisenia*). Hier stellte derselbe Autor fest, dass nach der Operation die Längsmuskulatur eine Veränderung erleidet, die als Dedifferenzierung zu bezeichnen ist: Die kontraktile Substanz degeneriert, die Kerne werden grösser, basophile Kernkörperchen treten auf und diese Zellen wandern auf die Ventralseite der Wunde. An der Wundstelle sind sie endgültig zu Regenerationszellen umgewandelt. Ebenso werden die Muskelzellen des Darmes und der Dissepimente umgewandelt. Die Herkunft des Regenerationsmaterials bei *Lumbriculus* und *Eisenia* ist dieselbe: Während aber bei den limikolen Formen (*Lumbriculus* und *Rhynchelmis*) im ausgewachsenen Wurme und bei Embryonen von *Eisenia* immer Neoblasten bereit

liegen, ist bei der erwachsenen *Eisemia* die Differenzierung bei der Ontogenese weiter gegangen, alle mesodermalen Elemente haben sich differenziert. Es muss also bei der Wundsetzung erst eine Rückdifferenzierung eintreten, die die mesodermalen Differenzierungen wieder auf einen embryonalen Zustand zurückführt, in dem sie dann als Regenerationszellen an der Wundstelle wirksam werden. Die besondere Grösse der Neoblasten führt Weitzmann auf ihre grosse Vermehrungsbereitschaft zurück.

In einer zweiten Arbeit hat Hämmerling (1930) seine an *Aeolosoma* erhobenen Befunde nach Untersuchungen an *Tubifex* zum grössten Teile selbst in Frage gestellt. Er verglich Embryonalentwicklung, Wachstum und Regeneration miteinander und kommt zu dem Resultat, dass ein Übertritt von Neoblasten in die Epidermis nicht nachzuweisen sei, sondern dass in dieser selbst eine Vermehrungszone liege, woraus sie ihr Zellmaterial beziehe. Aus umgewandelten Epidermiszellen werden auch Ringmuskeln und Borstensäcke gebildet, ebenso das Bauchmark. Damit ist die Aufgabe der Neoblasten auf die Bildung mesodermaler Gewebe beschränkt, da das Darmepithel sich selbst regeneriert. Aber auch für *Aeolosoma* lässt Hämmerling seine früheren Resultate nicht mehr voll gelten. Man kann also sagen, dass mit dieser Untersuchung Hämmerlings die Frage wieder auf den Stand der alten Autoren zur Jahrhundertswende zurückgeschoben worden war.

In dieser Situation sind Untersuchungen von Sayles (1927, 1928, 1931) von Bedeutung gewesen, die dartaten, dass die Formen der Kerne und der Nukleolen weitgehend auf Eingriffe an einer beliebigen Stelle des Wurmes (*Lumbriculus*) reagieren. In normalen Individuen sind die Kerne klein, nur in der Wachstumszone grösser, vor allem ventral. Ebenso sieht man grosse Nukleolen an den Borstensäcken in der Wachstumszone. Im Darm sind die Kernbestandteile in einer mittleren Region (20–30 Segmente vor dem Hinterende) auffälliger, als im übrigen Darmepithel. Dort im Mitteldarm fanden sich auch gelegentlich doppelte Nukleolen.

Sayles (1927) verglich damit nun das Verhalten der Kerne bei Regenerationsprozessen: Im Darm fand er doppelte Nukleolen und Mitosen 11–12 Segmente von der Wunde entfernt. Die Zellvermehrung dauert bis zum 6–7. Tage nach dem Eingriff. In unmittelbarer Umgebung der Wunde verändern auch Kerne und Nukleolen ihre Form, sie vergrössern sich ebenfalls. Aber Sayles meint, dass diese Veränderung nicht mit der Anwesenheit der Neoblasten in Verbindung zu bringen ist, sondern als ein selbständiger Vorgang anzusehen ist. Das Vorhandensein zweier Nukleolen in einem Kern ist nicht der Ausdruck für den Beginn einer mitotischen oder amitotischen Teilung, sondern bedeutet nur eine Zunahme der Nukleolarsubstanzen.

Ähnliche Resultate erzielte nun aber Sayles (1928) auch, wenn er die Gewebe von *Lumbriculus* mit hypotonischer Ringerlösung umspülte. Die gleichen Veränderungen im Darmepithel beobachtete Sayles (1931), wenn in die Leibeshöhle destilliertes Wasser injiziert wurde. Dagegen zeigten sich nach Injektion von isotonischer Ringerlösung keinerlei Veränderungen. Durch die Wundsetzung bei der Injektion kamen solche Veränderungen also nicht zustande.

Nach diesen Erfahrungen musste es aufschlussreich erscheinen, regenerative Vorgänge ohne Wundsetzung zu verfolgen und den Anteil der Neoblasten und des alten Gewebes bei der Neubildung festzustellen. Die von Sayles gesehenen Veränderungen wurden so ausgeschaltet. Dies gelang Stolte (1933 a) bei der Gattung *Dero*. Gegenüber den anderen Limikolen zeichnet sich *Dero* dadurch aus, dass die Regenerationsvorgänge sehr sparsam Zellmaterial verwenden. Daher lassen gerade die entscheidenden Frühstadien sich sehr gut herausfinden. Die Form der ungeschlechtlichen Fortpflanzung ist ja gewissermassen eine Regeneration mit nachfolgender Teilung. Aber bei diesem Regenerationsvorgang ist der Einfluss des umgebenden flüssigen Mediums völlig ausgeschaltet. In den frühesten Stadien sieht man ventrolateral vom Darm zwei Zentren grosser z. T. in mitotischer Teilung befindlicher Zellen, der Neoblasten. Ausserdem ist aber die Epidermis dieses Segmentes verdickt und mit ebenfalls vergrösserten Kernen dichter als die benachbarten Segmente besetzt. Die Verfolgung späterer Stadien lässt nun deutlich erkennen, dass der grösste Teil dieser Epidermiskerne eingewandert ist, dass aber darunter auch die ursprünglichen Epidermiskerne sind, die ihre Form verändert haben und dass Mitosen in diesem Stadium in der Epidermis völlig fehlen. Daraus ergibt sich aber, dass die Umwandlung der Epidermis durch Einflüsse erfolgt, die von aussen kommen: es ist eine Embryonalisierung des Ektoderms eingetreten, die von den Zentren der Neoblasten her induziert worden sein muss. Der Vorgang der Einwanderung von Zellen in die Epidermis wird aber vor allem dadurch deutlich, dass zwischen den Epidermiszellen zu Beginn Lücken sichtbar werden, die sich schliessen, wenn Zellen von innen her eingewandert sind (Fig. 10a-c). Nun beginnt der zweite Teil dieses Regenerationsvorganges: In der Epidermis treten Mitosen auf und Zellen treten über dem Darm aus um das Oberschlundganglion zu bilden. Bei diesem Vorgang bleibt das Entoderm anscheinend ganz unbeteiligt: kurz vor Beginn der Durchtrennung der beiden Zooide verdünnt sich das Darmepithel und reisst schliesslich durch. Die Wanderung der Neoblasten in der Teilungszone erfolgt in der Splanchnopleura, ausserhalb der Zone sieht man sie vor allem an den Gefässen entlang wandern, in erster Linie zwischen Bauchmark und Bauchgefäss.

Stolte hat schliesslich noch versucht, die divergierenden Ansichten über die Herkunft des Zellmaterials bei den Oligochäten einheitlich zu erklären mit dem Hinweis auf die Tatsache, dass die verschiedene Regenerationsintensität verschiedene histologische Bilder zustande kommen lässt. Für eine einwandfreie Beurteilung eignen sich nur ganz frühe Stadien solcher Neubildungen. Bei echten Regeneraten ist aber noch die Wirkung in Betracht zu ziehen, die der Eingriff als solcher an den Zellen der Wundregion hervorruft (Quellung, Vergrösserung und Verdoppelung der Nukleolen).

Auf zwei Wegen ist man ausser mit dem Regenerationsversuch der Materialfrage bei den Oligochäten ausserdem noch nachgegangen: Durch Beobachtung der Strahlenwirkung und mit dem Vergleich zwischen Neoblasten und den Geschlechtszellen.

Die Natur der Neoblasten erfährt eine weitere Aufhellung durch die Feststellung Eckerts (1930, 1934), dass bei der regulatorischen Resorption der

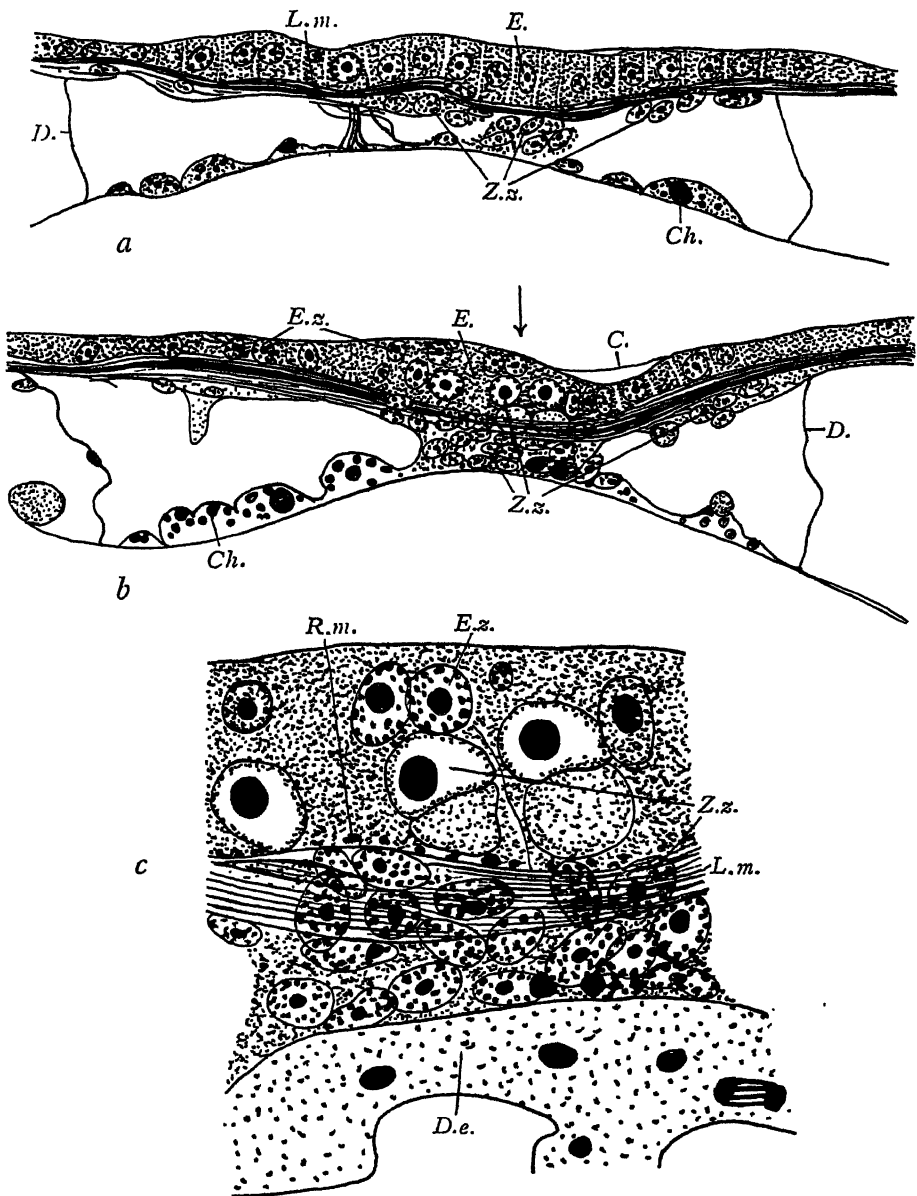


Fig. 10. *Dero limosa*. Medianer (a) und lateraler (b) Längsschnitt durch die dorsale Epidermis einer sehr frühen Teilungszone, 450 \times ; (c) = (b) bei \downarrow , 1350 \times (nach Stolte, 1933 a). C. Cuticula, Ch. Chloragogen, E. Epidermis, E.z. Epidermiszellen, D. Dissepiment, D.e. Darmepithel, L.m. Längsmuskeln, R.m. Ringmuskeln, Z.z. zuwandernde Zellen.

Teilungszone, etwa bei Hunger, die Neoblasten als erste einer Nekrotisierung verfallen an den Stellen, wo die aktivsten dieser Zellen liegen (Hinterende des Vorderzoids). Auch wirkt Nahrungsentziehung in der ersten Periode der Zonenanlage, während der das embryonale Material in der Zone vorherrscht, stärker, als in der zweiten mehr differenzierenden Periode.

Dass auch gegen Strahlung die Neoblasten empfindlicher sind als die anderen Körperzellen der Oligochäten, zeigte zum ersten Male Zhinkin (1932). Er bestrahlte *Lumbriculus variegatus* mit Röntgenstrahlen und fand, dass die Neoblasten am hinfälligsten waren. Waren sie ausgeschaltet, so segmentierte sich das Regenerat nicht mehr. Wird die Strahlendosis so gewählt, dass nicht alle Neoblasten zugrunde gehen, so entsteht ein depressives Regenerat. Fehlt infolgedessen das Mesoderm, dann bleibt das Regenerat auf einem niederen Zustande stehen. Wo die Neoblasten fehlen, entstehen auch keine Organe, die ihren Ursprung aus dem Ektoderm nehmen, wie z. B. das Nervensystem.

Ziemlich gleichzeitig hat Stone (1932, 1933) mit derselben Methode entsprechende Untersuchungen an *Tubifex* angestellt. Er fand zunächst, dass die Epidermis sich aus den in ihr vorkommenden Basalzellen regeneriert, das neue Nervensystem aus einer Epidermispartie nahe dem Bauchmark. Entoderm regeneriert aus den Basalzellen des alten Entoderms.

Mit Röntgenstrahlen bestrahlte normale Würmer besitzen keine Neoblasten mehr, nur ein kurzer Analkegel wird dann noch gebildet. Gibt man den Neoblasten Gelegenheit nach vorn zu wandern, ehe man sie bestrahlt, so sterben sie überall innerhalb 24 Stunden ab. Aber es können auch keine neuen Neoblasten aus dem Peritoneum gebildet werden, in einem Falle für einen Zeitraum von 147 Tagen. Auch die Regeneration von Ektoderm und Entoderm bleibt aus, da die Basalzellen durch die Röntgenstrahlen geschädigt sind. Undifferenzierte Reservezellen fand Stone am Hinterende von *Tubifex* nicht. Sie werden hier erst aus dem Peritoneum mobilisiert. Die durch die Röntgenstrahlen geschädigten Neoblasten werden durch Phagocyten beseitigt.

In der zweiten Arbeit berichtet Stone (1933) über entsprechende Versuche am Vorderende von *Tubifex*. Bei der normalen Regeneration werden Epidermis und Nervensystem vom Ektoderm aus, der Pharynx durch Umbildung des Darmvorderendes und die Muskellagen aus alten Muskelzellen gebildet. Es fehlt also vorn die Unterstützung der Neoblasten. Stone nimmt Fehlen richtender Reize an, da die Neoblasten auf den benachbarten Septen vorhanden sind. Aber auch diese Regeneration des Vorderendes wird durch Röntgenstrahlen völlig unterdrückt. Es fehlt dann die Zellproliferation in der Epidermis und die Bildung des Gehirns unterbleibt, das vordere Darmende endigt blind und die Muskulatur wächst darüber hinweg. Stone führt dieses Verhalten auf chemische Veränderungen in den Zellen zurück.

In einer weiteren Arbeit hat Zhinkin (1934) seine früheren Resultate bei *Rhynchelmis limosella* kontrolliert und bestätigt: Auch hier unterblieb eine Neubildung des Nervensystems, wenn die Neoblasten fehlten.

Im letzten Jahre haben noch zwei weitere Autoren die Strahlenwirkung auf die

Neoblasten untersucht: Turner (1934) sah bei *Lumbriculus inconstans* im normalen Regenerationsgeschehen Epidermis, Darm und Nervensystem aus den Basalzellen dieser epithelialen Schichten entstehen, die mesodermalen Gewebe aus den Neoblasten und ihren Abkommelingen. Wurden diese Gewebe aber einer Bestrahlung mit Röntgenstrahlen von 30 Minuten Dauer ausgesetzt, so waren die Basalzellen der Epithelien anscheinend reduziert oder zerstört, denn nun blieb Regeneration aus. Ebenso wenig wurden dann Borsten oder Nervensystem angelegt. Natürlich wurden auch die Neoblasten durch die Bestrahlung zerstört, während die differenzierten Zellen Schaden nicht leiden. Turner konstatierte auch während der Metamorphose der Neoblasten eine Zunahme der Mitochondrien in ihnen. Wundheilung erfolgt in unbestrahlten und bestrahlten Regeneraten, aber sie bedarf keiner mitotischen Vorgänge.

Rahm (1934) hat die Radiumstrahlen zu einer Prüfung der zellulären Vorgänge bei der ungeschlechtlichen Fortpflanzung der Naiden benutzt. Ausser in Schädigungen bestand die biologische Wirkung der Radiumstrahlen bei kleineren Dosen in Wachstumshemmung und Verzögerung der ungeschlechtlichen Fortpflanzung, kurze Bestrahlungszeiten konnten eine stimulierende Wirkung haben. Ehe die Wirkung sichtbar wird, verstreicht eine Latenzzeit, während deren die Differenzierungen weiter gehen. Durch die Radiumstrahlen werden die Blastocyten geschädigt oder vernichtet, im Darm bilden sich Lücken, die wohl auf degenerierende Zellen zurückzuführen sind. Die Degeneration beginnt immer in der Region der Blastocyten, also bei Würmern mit Zonen in der Teilungszone, bei zonenlosen Tieren am Hinterende. Andererseits sind ältere Tiere widerstandsfähiger als junge, da die ersteren weniger Blastocyten führen. Während bei starken Strahlendosen nach 4–5 Tagen Blastocyten im Naidenkörper nicht mehr festzustellen sind und die Würmer schliesslich eingehen, können bei schwächeren Dosen Würmer sich erholen, da noch Blastocyten überleben. Sie können das geschädigte Darmepithel wieder aufbauen und neues Zellmaterial für Wachstum und ungeschlechtliche Fortpflanzung liefern. An hochdifferenzierten Geweben, vor allem am Nervengewebe, waren niemals direkte Schädigungen durch die Radiumstrahlen nachzuweisen.

Schliesslich sei noch kurz auf die Beziehungen zwischen den Blastocyten und den Geschlechtszellen bei den Oligochäten hingewiesen. Ortmann (1921) spricht von latenten Genitalzellen, die bei *Nais* und *Lumbriculus* im Vorderkörper verteilt sind und als umgewandelte somatische Zellen anzusprechen seien, und Janda (1924) vermutet, dass die Regeneration der Geschlechtsorgane bei *Criodrilus lacuum* wahrscheinlich auf Peritonealzellen zurückzuführen ist.

Weitzmann (1928) fand keinen Unterschied in der Form der indifferenten Zellen und der Keimzellen bei *Lumbriculus*, sondern nur in der Zahl der Zellen. Er betont, dass in jedem beliebigen Segment indifferente Zellen zu Keimzellen werden können. Und schliesslich hat Stolte (1933 b) für *Stylaria lacustris* bewiesen, dass beim Übergang vom ungeschlechtlichen zum geschlechtlichen Zustande Neoblasten nach vorn wandern, sich wesentlich vergrössern und zu Keimzellen werden, dass hier also eine kontinuierliche Entwicklungsreihe vorliegt. Da die Geschlechtsorgane in einer festen topographischen Beziehung zur Teilungszone stehen, diese

aber von Aussenfaktoren in ihrer Lage bestimmt wird, so ist damit auch die Lage der Gonaden wechselnd, also eine frühe Anlage von Urkeimzellen ausgeschlossen. Die Blastocyten sind also das Ausgangsmaterial einerseits für alle Wachstumsvorgänge einschliesslich der Regeneration, anderseits für die geschlechtliche Fortpflanzung.

Im Gegensatz zu dem Verhalten der meisten Oligochäten konnte bei der Gattung *Branchiobdella* von Janda (1928) nur Verschluss der Wunde beobachtet werden.

In neuester Zeit sind die Neoblasten bis in die embryonalen Periode verfolgt worden, P. S. Chen (1934) hat den Regenerationsvorgang bei Embryonen und jungen Würmchen von *Tubifex* verfolgt. Er fand, dass die Epidermis in diesem Entwicklungszustande noch sehr grosse Zellen besitzt und in ihr Mitosen häufig sind, sodass schwer zu entscheiden war, ob sie von sich aus oder durch Neoblasten ihren Neuaufbau erfährt. Das Entoderm regeneriert sich selbst, nach auftretenden Mitosen zu urteilen, das Mesoderm wird durch die Neoblastenabkömmlinge neu gebildet. Das neue Nervensystem wird aus der Epidermis angelegt. Von dort wandern die Zellen aus und vermehren sich dann an Ort und Stelle sehr lebhaft.

Penners (1934) hat mittels ultravioletten Lichts am Keim von *Tubifex* die Teloblasten ausgeschaltet und damit wahrscheinlich die Quelle für die Bildung der Neoblasten unterbunden. Es kam zur Anlage eines Bauchmarks, auch dann, wenn die Telektoblasten durch Bestrahlung entfernt waren. Wurden die beiden Urmesodermzellen auf die gleiche Weise entfernt, so wuchs später Mesodermmaterial vom Stumpf her nach hinten und schuf dort eine neue Wachstumszone. Wurden aber sowohl die Urmesodermzellen wie die acht Telektoblasten beseitigt, so ging trotzdem die Entwicklung des ganzen Keimstreifs normal weiter und das Entoderm segmentierte sich nach Massgabe des vorhandenen Keimstreifrestes. Die interessante Feststellung der Bildung eines Bauchmarks in einem vom ektodermalen Keimstreif freien Hinterende ist noch nicht geklärt.

Zusammenfassung. Die Neoblasten der Oligochäten sind seit Randolph (1892) bekannt. Sie scheinen für alle Organe mit Ausnahme des Darmes die Grundlage der Regeneration zu bilden. Allerdings treten dazu auch Zellen vom Charakter der Basal- und Peritonealzellen. Alle zusammen kann man als Blastocyten (Stolte) bezeichnen. Die Neoblastenform herrscht bei den Limikolen vor, die Form der Peritonealzellen bei den Terrikolen. Die differenzierten Gewebe können sowohl durch die Neoblasten wie durch Änderungen des Mediums (Sayles) embryonalisiert werden. Strahlenwirkung schaltet zuerst die Neoblasten aus, Regeneration wird dann unterdrückt oder verzögert. Wie für die Körperzellen, so bilden auch für die Keimzellen die Neoblasten die Grundlage. Diese totipotenten Zellen lassen sich mit grosser Wahrscheinlichkeit auf die Urmesodermzellen des Keimes zurückführen.

VIII. GEPHYREA.

Über die Herkunft des Regenerationsmaterials von *Phascolion strombi* (Sipunculidae) unterrichtet eine Arbeit von Schleip (1934a). Er untersuchte die Regeneration des Rüssels.

Schleip fand unter dem unsegmentierten Bauchmark einen Zellstrang aus viel grösseren Zellen, als es die Ganglienzellen sind, sich hinziehen, in den einzelnen Körperabschnitten von verschiedener Mächtigkeit (Fig. 11). Er bezeichnet ihn als Regenerationsstrang und nimmt eine ektodermale Herkunft an. Dieser Strang ist mit dem Bauchmark zusammen von Peritoneum umhüllt.

Als Quellen des Regenerationsgewebes gibt Schleip Ektoderm und Mesoderm an. Mesenchym erscheint in Gestalt von Amöbocyten schon am 1. Tage nach dem Eingriff, vor allem an dem Rüsselstumpf. Hier bilden die Zellen eine dünne Membran. Dieses mesodermale Material bildet die Brücke zwischen dem alten Stück des

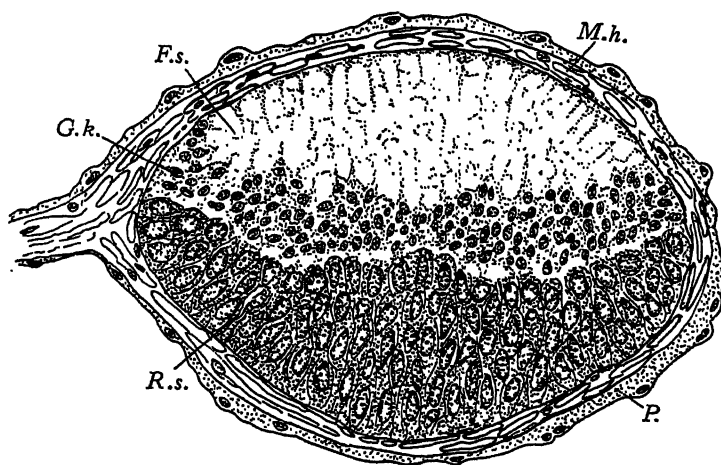


Fig. 11. *Phascolion strombi*. Querschnitt durch das Bauchmark mit Regenerationsstrang aus der Mitte des vorderen Rumpfdrittels (nach Schleip, 1934a). F.s. Fasersubstanz, G.k. Ganglienzellkerne, M.h. muskulöse Hülle, P. Peritoneum, R.s. Regenerationsstrang.

Rüssels und dem Regenerat. Das ektodermale Material liefert der Regenerationsstrang und zwar immer vom hinteren Bauchmarkstumpf her. Schon im Laufe des ersten Tages beginnt dieser Prozess: Zellen legen sich nun in immer stärkerer Masse vor die Rüsselstumpffläche, auf die der Regenerationszellstrang umbiegt. Während dieser ersten Tagen fehlen Mitosen sowohl im Ektoderm wie im Mesoderm. Bis zum 4. Tage ist dann meistens die Neubildung des fehlenden Rüssels in der Anlage beendet. Nun treten auch Mitosen auf. Schleip vertritt die Ansicht, dass die Anlage durch Zuwanderung von Zellmaterial gebildet wird, das kontinuierlich aus dem Regenerationsstrang in die neue Rüsselanlage übergeht. Bei regenerierenden Tieren ist der Strang im Rüssel nämlich relativ mächtig gegenüber den Befunden bei nichtregenerierten Tieren. In der weiteren Ausgestaltung des Regenerates liefert das Ektoderm Epithel und Nervensystem, das Mesoderm Muskulatur, Bindegewebe und Peritoneum. Da das ektodermale Zellmaterial innerhalb einer Amöbo-

cytenansammlung vorwächst, kann man einen Einfluss der mesodermalen auf die ektodermale Anlage annehmen. Diese Untersuchung an *Phascolion* zeigt also, dass dieser Vertreter im Gegensatz zu den Anneliden ektodermale und mesodermale Regenerationszellen besitzt.

Eine entsprechende Untersuchung wurde von Schleip (1934b) an *Phascolosoma minutum* angestellt. Hier kommen in den basalen Teilen des Bauchmarks ebenfalls Regenerationszellen vor, wenn auch nicht so zahlreich und nicht in geschlossenem Verbands. Dort sind ektodermale und mesodermale Zellen nur noch schwer zu unterscheiden. Dieser Regenerationsvorgang läuft aber auch ab, wenn nicht das Bauchmark, sondern nur andere Teile des Organismus verletzt sind: auch dann wandern Regenerationszellen aus dem Bauchmark in das mesodermale Gewebe an der Wundstelle ein. Im Bauchmark normaler Tiere wurden Übergangsformen der Kerne von Regenerations- und Ganglienzellen gefunden. Daraus schliesst Schleip, dass ektodermale Neoblasten auch im normalen Lebenszyklus neue Ganglienzellen liefern und also auch für das Wachstum Leistungen vollbringen.

IX. BRYOZOA.

Faulkner (1933) untersuchte bei *Alcyonidium gelatinosum* das Verhältnis von somatischen Zellen zu den Keimzellen. Faulkner fand in der Region der Anheftung und Zellvermehrung Zellen, die sie als Neoblasten bezeichnet. Sie wandern zwischen die beiden Zellagen eines Polypen und bilden dort eine "Neoblastenmorula". Sie sind wohl epithelialen Ursprungs und bleiben zunächst undifferenziert. Später steuern sie zur Bildung des Darmes bei und wandern z. T. schliesslich in das Darmepithel ein, ohne aber ihre Neoblastennatur zu verlieren. Beim Übergang zum geschlechtlichen Zustand gehen die weiblichen Keimzellen ebenfalls aus der Neoblastenmorula hervor, während diese Bildung bei den ungeschlechtlichen Individuen allmählich schwindet. Nach ihrer interepithelialen Lage (zwischen Basalmembran des Entoderms und dem Peritoneum), ihrem undifferenzierten Zustande und nach ihrem Eingreifen in somatische und germinative Vorgänge müssen diese Zellen als Neoblasten bezeichnet werden.

X. TUNICATA.

Schaxel (1914) schilderte bei *Clavelina* die Reduktion sämtlicher Organe und deren Wiederaufbau durch indifferente Reservezellen, die in den ekto- und entodermalen Epithelien und in dem Mesenchym der Leibeshöhle liegen. Sie sind im Epithel etwa kubisch mit sehr klarem Plasma, einem grossen Kern und sehr kleinem Nukleolus. In der Leibeshöhle haben sie mehr das Aussehen von embryonalen Mesenchymzellen.

Später hat Spek (1927) bei *Clavelina lepadiformis* durch Vitalfarbstoffe sog. Tropfenzellen sichtbar gemacht, die überall in den Geweben verteilt sind. Sie umschliessen einen runden bis länglichen eiweisshaltigen Körper, der sich in Neutralrot tiefrot, in Nilblausulfat blau färbt. Diese Zellen entsprechen den Mesenchymzellen (Fig. 12). Sie liegen zu Schnüren gereiht unter zahlreichen

Organen oder treiben auch im Blute. Im ungestörten Organismus liegen sie vielfach unter den Epithelien und kriechen wohl auch zwischen die Epithelzellen.

Diese Zellen wandern in die Winterknospen ein, die unter ungünstigen Aussenbedingungen sich entwickeln können. Sie liegen dann gewissermassen in einem Epithelsack zusammen und verschmelzen meist zu grösseren Protoplasmahaufen. Beim Erwachen der Winterknospe wandern Tropfenzellen zunächst in das Epithel ein, dann bilden sich durch Aneinanderlagerung von Zellpaketen die anderen Organe und so wird in drei Tagen ein neuer Organismus aufgebaut. Bei geringeren Reduktionen werden die reduzierten Gewebe ebenfalls wieder durch die Tropfenzellen erneuert.

In den Ovarien kriechen die Tropfenzellen auf die heranreifenden Eizellen zu und schmiegen sich ihrer Oberfläche dicht an. Schliesslich liegt ein ganzer Ring solcher Zellen um die Eizelle herum. Aus dem Nachlassen der Rotfärbung der

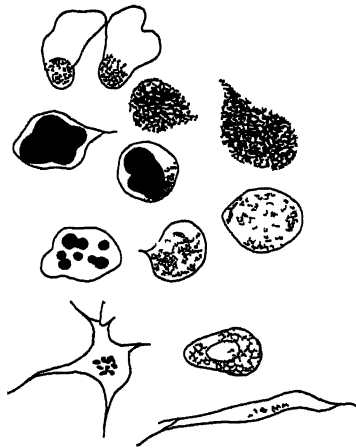


Fig 12 *Clavelina lepadiformis*. Amoboide Zellelemente aus dem Bindegewebe (nach Spek, 1927).

angelagerten Zellen kann man schliessen, dass erhebliche Eiweissmengen aus den Tropfenzellen an die Eizellen abgegeben werden. Dabei wandern die Zellen vorübergehend in die Eizellen hinein. In der Folge werden sie zu den bekannten Testazellen.

Ob auch diese Tropfenzellen in das Keimepithel einwandern, muss zunächst noch offen bleiben.

Bei Regeneration junger Tiere konnte Spek beobachten, dass um die Schnittwunde eine bedeutende Ansammlung von Tropfenzellen in der Tunika zustande kam. Auch für den Aufbau des Ektoderms werden zahlreiche Tropfenzellen verwendet, dagegen verläuft die Regeneration entodermaler Organe völlig ohne Beteiligung dieser Zellen. Allerdings muss nach Spek hier zwischen einer ersten formativen und einer späteren nutritiven Rolle der Zellen geschieden werden: die letztere ist auch im Entoderm sehr wohl zu erkennen, aber erst nachdem z. B. der Kiemenkorb fertig ausgebildet ist.

Die Reduktionsvorgänge, die schon Schaxel schilderte, lassen die Tropfen-

zellen unberührt. Die Herkunft der Tropfenzellen lässt sich noch nicht klar erkennen. Nur konnte Spek feststellen, dass in den Mitteldarmzellen die färbbare Substanz, die die Eiweissreaktionen gibt, durch Resorption aus der Nahrung ausgeschieden wird. Die von Schaxel beobachteten Phagocyten hält Spek für identisch mit den Tropfenzellen.

In neuester Zeit hat Brien (1930) wiederum zu der Frage Stellung genommen. Er nimmt an, dass die omnipotenten Zellen mesenchymatischen Ursprungs sind. Von ihnen gehen alle Neubildungen aus, vorher fehlt ihnen jede histologische Differenzierung, sie besitzen einen grossen Kern mit deutlichem Nukleolus und reichlich basophiles Plasma. Die Tropfenzellen Speks dagegen sollen nur eine trophische Bedeutung haben. Derselbe Autor (1933) gibt aber für *Archiaseidia neapolitana* an, dass das Epikard, ein Abkömmling des Entoderms, für die Regeneration verantwortlich ist. Nur Oesophagus und Nervensystem regenerieren selbständig, das letztere aus dem sog. Dorsalstrang, einem Zellstrang embryonalen Charakters. Diese Frage bedarf also noch der weiteren Untersuchung.

Weigand (1930) bestrahlte *Clavelina lepadiformis* mit Radiumstrahlen, aber niemals konnte Bestrahlung die Regeneration zum Stillstand bringen. Auch er fand, dass die Tropfenzellen für die Regeneration des Entoderms nicht in Frage kommen. Wie allerdings die Regeneration des Kiemenkorbes z. B. vor sich geht, konnte nicht geklärt werden. Die geringe Wirkung der Radiumstrahlung führt Weigand auf das späte Auftreten von Mitosen in den Geweben von *Clavelina* zurück. Am Kiemenkorb gibt sich die Radiumwirkung im Auftreten abnormer Mitosen zu erkennen. Über die Herkunft des Regenerationsmaterials konnte Weigand keine Aussagen machen.

v. Haffner (1933) hat bei seinen Beobachtungen über die Anlage überzähliger Ocellen bei *Ciona intestinalis* feststellen können, dass die Neubildung der Gewebe mit grosser Wahrscheinlichkeit auf mesenchymatische Blutzellen zurückzuführen ist. Ein Teil dieser Zellen durchwandert die Epidermis und wird zu Mantelzellen, ein anderer Teil wird durch Ausbildung gelbroten Pigmentes zu Pigmentzellen, die sich später zu einem Pigmentmantel für die Ocellen zusammenballen.

Zusammenfassung. Der Ursprung des Regenerationsmaterials der Tunikaten wird bisher teils im Mesenchym, teils im Entoderm gesucht. Eine Einigung, welche Zellform verantwortlich ist, konnte noch nicht erzielt werden.

XI. DIE ÜBRIGEN GRUPPEN DER WIRBELLOSEN.

Arthropoda. Bordage (1905) sah die Muskeln der Extremitäten der Phasmide *Rhaphiderus* aus dem Mesenchym nach einer Periode der Degeneration sich neu bilden.

Ost (1906) bemerkte bei *Oniscus murarius* als ersten Wundverschluss einen Pfropf von Blutgerinnsel und allerlei Geweberesten. Später wuchs Hypodermis von beiden Seiten über die Wunde. Muskeln und Drüsen regenerieren vom Ektoderm, Nerven vom Stumpf her. Wege (1911) kommt für *Asellus aquaticus* zu einer ganz ähnlichen Ansicht. Auch Heldmann (1929) hat bei *Dixippus morosus*

als ersten Wundverschluss einen Blutpfropf gesehen; den definitiven Wundverschluss bildete Hypodermis. Alle übrigen Organe wurden so gebildet, wie schon die früheren Autoren angaben, nur die Muskulatur soll aus dem Fettkörper und seinen Wanderzellen aufgebaut werden. Friedrich (1930) beobachtete am gleichen Material, dass von der Hypodermis aus Zellen ins Innere wuchern und sich z. B. zwischen die Muskelendstücke schieben, die durch formative Reize das Material zu Muskulatur differenzieren lassen. Bei Collembolen hat Boelitz (1933) die Regeneration des Mitteldarmepithels aus basalen Zellen (Regenerationszellen) nachgewiesen. Diese Zellen liegen zwischen Basalmembran und den funktionierenden Darmzellen. Die verbrauchten Epithelzellen werden als Ganzes nach innen abgestossen.

Mollusca. M. Lange (1920) untersuchte Wundheilung und Regeneration der Cephalopodenarme. Auch hier erfolgt der erste Wundverschluss durch Blutgerinnsel. Über die Wunde kriecht nach 24 Stunden flaches Epithel und überdeckt sie. Später nimmt es allmählich die normale Höhe ein und schliesslich entwickelt sich ein kleiner Regenerationskegel. Muskulatur und Nervensystem machen eine Dedifferenzierung durch, z. T. mit Hilfe von Phagocyten und werden durch Sarco- bzw. Neuroblasten wiederaufgebaut. Nach Lange sollen die Neuroblasten von der Neuroglia und den Ganglien stammen. Das neue Bindegewebe soll sich aus angesammelten Blutkörperchen im Blastem entwickeln.

Echinoderma. Über das Regenerationsmaterial der Echinodermen berichten nur wenige Untersucher. Zirpolo (1928) meint, dass bei *Asterias* überall totipotenten Regenerationsblastem im Körper vorhanden ist. Bei den Holothuriern hat Bertolini (1930, 1933) die Regeneration des ausgeworfenen Darmes histologisch untersucht. Bei *Stichopus regalis* wird der Darm durch das benachbarte Mesenchym gebildet, bei *Holothuria* dagegen von den beiden Stümpfen des Darmes her. Diesen Unterschied der Bildung führt der Verfasser darauf zurück, dass bei *Stichopus* der Darm periodisch ausgestossen wird und die Neubildung, gewissermassen vorgesehen, sehr schnell erfolgt, während das Ausstossen des Darmes bei *Holothuria* nur gelegentlich erfolgt.

XII. BESPRECHUNG DER ERGEBNISSE.

Betrachtet man die Ergebnisse der vorstehenden Zusammenstellung, so ist leicht zu erkennen, dass die Frage nach der Materialherkunft bei Regeneration zu den verschiedenen Zeiten der Beschäftigung mit dem Regenerationsproblem in recht wechselnder Weise beantwortet worden ist.

Man kann wohl sagen, dass nach der ältesten Auffassung die Körperzellen des Regenerationsstumpfes für den Wiederaufbau des Fehlenden sorgen und jede Gewebeart für die Lieferung des Gleichen verantwortlich ist. Diese Ansicht wurde gestützt durch die allgemeine Anschauung, dass die Keimblattgrenzen durch Lieferung von Zellmaterial an eine andere Gewebeart nicht überschritten werden könnten. Diese so charakterisierte Auffassung hat in Hämmerling (1930) noch einen späten Vertreter gefunden. Ausserdem mögen bei dem damals vorwiegend morphologischen Interesse am Regenerationsproblem sehr oft erst späte Stadien, in denen die Differenzierung des Materials schon begonnen hatte, betrachtet worden

sein. Die für unsere Frage wichtigen histologischen Bilder lassen sich aber am besten in den Frühstadien finden.

Eine andere ebenso alte Meinung spricht dem Ektoderm die Aufgabe zu, über seine Keimblattgrenze hinweg Zellmaterial an das Mesoderm abzugeben, um dort mesodermale Organe aufzubauen. Diese Zellbewegungen wurden aber an Regionen abgelesen, die durch den Regenerationseingriff in ihrem Aussehen verändert waren oder sie wurden in Wachstumszonen am Hinterende angenommen, in denen die definitive Ausbildung der Gewebe noch nicht beendet war. Die Selbständigkeit des regenerierenden Entoderms ist dagegen schon frühzeitig behauptet und in der Folge immer wieder bestätigt worden.

Seitdem aber Randolph (1892) auf indifferente Zellen (Neoblasten) aufmerksam gemacht hat, ist nun die Bedeutung solcher selbständiger Zellen in den letzten Jahren immer stärker erkannt worden, nicht allein in experimentell erzeugten Regeneraten, sondern auch im normalen Geschehen der ungeschlechtlichen Vermehrung und bei der Entwicklung aus Ruhestadien. Vor allem aber haben die Ausschaltungsversuche durch Strahlenwirkung gezeigt, dass gegen diese die undifferenzierten Zellen besonders empfindlich sind, wie bei Cölenteraten, Turbellarien, Oligochäten und Tunicaten nachzuweisen war. Es ist aber als besonders wichtig zu betonen, dass durch die Bestrahlung nicht nur die freien undifferenzierten Zellen ausgeschaltet wurden, sondern auch Zellen in der Epidermis und dem Darmepithel, die als Basalzellen bezeichnet werden, und die man als Ersatzzellen betrachten kann. Es liegt nahe sie als Abkömmlinge mesodermaler formativer Zellen anzusehen. Diese Frage bedarf noch weiterer Klärung.

Dass aber solche Übergangsformen zwischen undifferenzierten Zellen und den differenzierten Epithelien ektodermalen, mesodermalen und entodermalen Ursprungs vorkommen, geht aus der Tatsache hervor, dass unter natürlichen Bedingungen, also vor dem experimentellen Eingriff, bei vielen Formen keine Zellkomplexe sichtbar sind, die deutlich als undifferenzierte Zellen vom Charakter der Neoblasten zu erkennen wären, dass sie nach dem Eingriff aber zahlreich auftreten können. Das berechtigt zu dem Schluss, dass schon auf dem Wege der Differenzierung begriffene (oder ruhende), überwiegend mesenchymale Zellen gegebenenfalls durch Dedifferenzierung zu totipotenten Zellen werden können. Wie ja auch Entodermzellen (Sayles) und Epidermiszellen (Stolte) diesen Charakter kurz nach dem Eingriff oder unter Veränderungen des inneren Mediums annehmen können.

Daraus ergibt sich folgende *Gesamtaufassung über die Herkunft des Regenerationsmaterials bei den Wirbellosen*: In den drei Keimblättern (Ekto- Meso- und Entoderm) haben wir drei Regionen vor uns, von denen jede in der Differenzierung ihrer Zellen verschieden weit fortgeschritten ist. Im Ektoderm ist diese Differenzierung am weitesten gekommen: Seine Wiederherstellung im Regenerat ist ohne Zuhilfenahme relativ undifferenzierter Zellen kaum möglich. Dagegen ist der Gestaltwechsel der Zellen dieser Körperschicht für die Wundheilung von grösster Bedeutung. Demgegenüber ist das Entoderm weit weniger differenziert, sodass die Regeneration des Darmes meist vom Stumpf ausgeht. Allerdings bilden die Basalzellen eine Reserve, die unauffällig diesem System undifferenzierte Zellen zuführen kann. Das

Mesoderm (Mesepithel, Mesenchym) schliesslich ist die am wenigsten differenzierte Schicht, aus der Zellen hervorgehen können, die sowohl dem Ektoderm wie dem Entoderm Material liefern und die mesodermalen Organe aufbauen. Diese Zellen können entweder in der Form von Peritonealzellen ein relativ unscheinbares Aussehen haben, oder sie sind als Neoblasten schon im ungestörten Organismus deutlich zu erkennen. Wann der eine oder der andere Zustand auftritt und wie man sich Tätigkeit und Wirkung der Neoblasten vorzustellen hat, das sind Fragen, die an dieser Stelle nicht besprochen werden sollen.

XIII. ALLGEMEINE ZUSAMMENFASSUNG.

1. Die Herkunft des Regenerationsmaterials wurde bisher bei den Poriferen, Coelenteraten, Turbellarien, Nemertinen, Polychäten, Oligochäten, Sipunculoiden, Bryozoen und Tunikaten, sowie in einzelnen Fällen auch bei Arthropoden, Mollusken und Echinodermen verfolgt.
2. Die Wundheilung geschieht in den meisten Fällen durch Überdeckung der Wunde mit der Epidermis, deren Zellen aktiv darüber hinwegwandern, manchmal unterstützt durch von unten einwandernde mesenchymale Zellen.
3. Unter den drei Keimblättern ist das Entoderm das selbständigste. Der Darm wird fast immer vom Stumpf aus regeneriert, wahrscheinlich unter Benutzung der Basalzellen. Wo kein Stumpf vorhanden ist, kann seine Bildung manchmal vom Mesenchym aus erfolgen.
4. Das Ektoderm ist bei der Regeneration auf die Unterstützung durch totipotente Zellen angewiesen, unter deren Wirkung vielfach eine Embryonalisierung der Epidermis erfolgt. Das Ektoderm ist also die am weitesten differenzierte Körperschicht.
5. Das Mesoderm (s. l.) ist das am wenigsten differenzierte Keimblatt und liefert in vielen Fällen totipotente oder dedifferenzierte Zellen, die zuweilen alle Organe wiederherstellen können.
6. Die totipotenten Zellen treten entweder in der Form der Neoblasten oder als Basalzellen, vielleicht auch als Peritonealzellen auf. Alle diese Formen von Regenerationszellen können als Blastocyten (Stolte) zusammengefasst werden.
7. Die Schwämme besitzen zahlreiche Zellformen, die, künstlich getrennt, nach teilweiser Dedifferenzierung die Organe wieder aufbauen. Die totipotenten Zellen sind hier die Archäocyten.
8. Wo ein Mesoderm fehlt, wie bei den Coelenteraten, liegen die sog. interstitiellen Zellen im Ektoderm, können aber auch in das Entoderm überwandern.
9. Für die Tunikaten ist noch nicht entschieden, ob die Regenerate von mesenchymalen Zellen, speziell von den sog. Tropfenzellen (Spek) aufgebaut werden.
10. Bei den höher differenzierten Tiergruppen der Arthropoden, Mollusken und Echinodermen ist über die Herkunft des regenerativen Zellmaterials bisher nur wenig bekannt geworden. Die gute Regenerationsfähigkeit der Asteroiden, Ophiuriden und Holothurien unter den Echinodermen lässt vermuten, dass in diesen Organismen überall Zellreserven zur Verfügung stehen.

XIV. SUMMARY.

1. The origin of regenerative material has been studied in sponges, coelenterates, turbellarians, nemerteans, polychaetes, oligochaetes, sipunculids, polyzoa and tunicates, and in a few instances also in arthropods, molluscs and echinoderms.
2. In most cases the healing of a wound is accomplished by the active migration of epidermal cells, often aided by mesenchyme cells moving up from beneath.
3. Of the three germ layers, the endoderm is the most independent. The gut is almost always regenerated from its stump, probably with the help of basal cells. When there is no stump, the formation of the gut may be sometimes accomplished by mesenchyme.
4. The ectoderm, which is the most differentiated germ layer, is assisted in regeneration by totipotent cells, which to a great extent render the epidermis embryonic.
5. The mesoderm is the least differentiated germ layer and in many cases it furnishes totipotent, or dedifferentiated, cells which in certain cases can regenerate all organs.
6. The totipotent cells take the form either of neoblasts or of basal cells, and perhaps also of peritoneal cells. All these types of regenerative cells may be called blastocysts.
7. Sponges possess many sorts of cells, which, when artificially separated from one another, can reconstitute the organs, after they have undergone partial dedifferentiation. In this case the archaeocytes are the totipotent cells.
8. When there is no mesoderm, as in the coelenterates, the so-called interstitial cells lie in the ectoderm, but can also migrate into the endoderm.
9. In tunicates it has not yet been decided whether or not regeneration is accomplished by mesenchyme cells, and particularly by the so-called "drop-cells".
10. Little is known concerning the cellular basis of regeneration in the more highly differentiated groups of arthropods, molluscs and echinoderms.

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ADDENDUM

Während der Drucklegung erschienen zwei weitere Arbeiten, die die vorstehenden Fragen behandeln: A. A. Wolsky (1935) (*Nature*, Lond., 135, 102) untersuchte die Regenerate hungernder *Dendrocoelum* und fand, dass die Regenerationsfähigkeit durch Hunger herabgedrückt wurde, was für den entscheidenden Einfluss der Regenerationszellen spricht. V. Janda (1935) (*Zool. Anz.* 110, 291) bestätigte im wesentlichen die Ergebnisse von Zhinkin und Rahm durch die Prüfung des Einflusses von Radiumstrahlen auf reparative Vorgänge bei *Criodrilus lacuum*, *Rhynchelmis limosella* und *Lumbriculus variegatus*.

THE RETENTION AND PHYSIOLOGICAL ROLE OF UREA IN THE ELASMOBRANCHII

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I. INTRODUCTION.

THE elasmobranch or cartilaginous fishes are unique in normally possessing in the blood, the body fluids and the tissues large quantities of urea, which may be present in amounts exceeding 2.5 per cent. Since in other animals urea is a waste product formed by the degradation of protein nitrogen, and as such is excreted from the body as rapidly as it is formed, this phenomenon of retention by the elasmobranchs is of considerable interest.

The term "uraemia" has long been used to denote a condition of elevated blood urea in man or other mammals, due to renal insufficiency; although it is now accepted that the pathological consequences of this condition are not due to the urea itself, nevertheless the term has such a definite pathological connotation that it seems inadvisable to apply it without qualification in this instance. For it is established beyond doubt that the retention of urea in the elasmobranchs is a consequence of a normal physiological process, and therefore it is not to be confused with the pathological retention occasionally observed in the higher animals.

Excluding the Cyclostomata (or jawless fishes, which are probably degenerate ostracoderms), the elasmobranchs comprise the lowest division of the piscine world.

The class Pisces is divided by most authorities into the subclasses, Elasmobranchii (or Selachii) and the Teleostomi, the latter being made up for the most part by the true bony fishes. The Elasmobranchii in turn are divided into three principal orders: the Chimaeroidei (rat fishes, elephant fishes, etc.), the Selachii (sharks and dogfishes) and the Batoidei (sawfishes, rays and skates). These are typically marine and most abundant in tropical and subtropical waters, but many of them ascend rivers beyond influence of the tides, and a few are permanent inhabitants of fresh water. All three orders are of great antiquity, the ancestral forms having apparently differentiated from each other and from the parent stem in the late Silurian or early Devonian periods.

Elevated blood urea is shared by all orders of the Elasmobranchii, and in view of this fact, in view of the antiquity of the subclass as a whole, and in view of certain morphological and reproductive specialisations that appear to be associated with this biochemical peculiarity, the interpretation of this urea retention should be of interest to the palaeontologist and the ichthyologist, as well as to the biochemist and physiologist.

II. THE DISTRIBUTION OF UREA IN THE ELASMOBRANCH FISHES.

In the course of investigations on the nitrogenous constituents of man and animals, Städeler and Frérichs (1858) discovered that extracts of the muscles of the skates, *Raja batis* and *R. clavata*, and of the dogfish, *Scyllium canicula*, contained such "colossal quantities" of urea that the syrup solidified to a solid mass upon the addition of an equal volume of nitric acid. Examination of other animals, including some teleosts and *Petromyzon*, revealed at most only traces of this substance. The next year Städeler (1859) extended this observation to include the spiny dogfish, *Spinax acanthias*, the torpedos, *Torpedo marmorata* and *T. ocellata*, and it was confirmed by Schultze (1861) and Rabuteau and Papillon (1873).

The first to undertake a systematic examination of the distribution of urea in the vertebrates was Krukenberg. In 1881 he reported that he could recover large amounts of this substance from *Scyllium canicula*, *Mustelus vulgaris*, *M. laevis*, *Acanthias vulgaris*, *Squatina vulgaris*, *Torpedo marmorata* and *Myliobatis aquila*, but was unable to obtain it from *Amphioxus*, *Anmocoetes*, *Petromyzon*, *Acipenser*, *Conger*, *Cyprinus*, *Crenilabrus*, *Perca*, *Trygla*, *Thynnus*, *Pelamys*, *Luvourus*, *Caranx*, *Lubia*, *Lophius*, *Rana*, or *Testudo*. Later (1886, 1887, 1888) he reported that urea was present in large amounts in the sawfish *Pristis* and in *Chimaera monstrosus*, but absent in the lungfish *Neoceratodus*. The last two observations were particularly significant because the Chimaeroidei are so closely related to the sharks and rays that they are usually included in the subclass Elasmobranchii, whereas the lungfish, *Neoceratodus*, although an extremely primitive form, is more closely affiliated with the higher bony fishes. More recently, Hunter (1929) has remarked, in connection with observations on the distribution of arginase in fishes, that "the presence of pre-formed urea in all rat fish (*Hydrolagus colliei*) necessitated a special control for each determination". (We may include here a previously unpublished analysis of this fish from La Jolla, California, a specimen of which was preserved in alcohol for us

immediately after death. The alcohol and tissues were analysed *en masse* and sufficient urea was found to yield 2.4 per cent. of the original body weight. No ammonia was present, indicating that no decomposition had occurred.) Dakin (1931) has reported 2.4–2.86 per cent. of urea in the blood of *Callorhynchus millii*. These observations indicate that urea is as universally present in the Chimaeroidei as in the Selachii and Batoidei.

Krukenberg also showed that the young embryo of *Mustelus laevis* and *Acanthias vulgaris*, and the egg-yolk of *Scyllium canicula*, *Myliobatis aquila*, *Pristis antiquorum* and *Torpedo ocellata* contained large amounts of urea. (Also see section XI and Needham and Needham, 1930.) The presence of urea in the egg and embryo, together with an apparently irregular distribution of this substance in the muscles of the adult, led Krukenberg to assert that the tissues of the Elasmobranchii had a special affinity for this substance. He supposed that the urea existed in some state of chemical combination that was decomposed by even such mild treatment as maceration and water extraction and, in the light of this theory, he attempted to correlate the urea content of various tissues with their activity and function.

In 1890 von Schroeder attacked the problem in a more quantitative manner. He showed that the blood of *Scyllium canicula* contained 2.61 per cent. urea, the muscle 1.95 and the liver 1.36 per cent. When corrected for water content these figures were 2.95, 2.41, and 2.67 gm. per 100 gm. water. He pointed out that when the plasma contained so much urea it is not remarkable that the other organs are proportionally rich, and that it is unnecessary to assume any specific attractive power on the part of the tissues. The extirpation of the liver had no influence on the urea content of the muscle, and von Schroeder resorted to a theory of renal insufficiency, supposing that the kidney excreted the urea only with difficulty, *i.e.* if the blood contained a proportionally large amount, and he likened the uraemia of the elasmobranchs to pathological uraemia in man.

Krukenberg (1888) had published figures showing that the electric organ of *Torpedo ocellata* might contain nearly twice as much urea as did muscle, whereas the analyses of Marcuse (1891) indicated a more nearly uniform distribution; Gréhant and Jolyet (1891) were led by these observations to make further analyses in relation to excitation, using the denervated electric organ on one side as a control. They found that electric discharge was accompanied by a considerable increase in urea content. In one instance this was 1.38 per cent. on the excited side, in contrast to 0.74 per cent. on the control; in a second instance these figures were 2.66 per cent. and 0.89 and, in a third, 1.15 and 0.57. Two years later Röhmman (1893) re-examined this point; he asserted that, although there was a marked increase of acidity in the excited electric organ, in contradiction to Marcuse's observations, he confirmed Marcuse in respect to urea content, finding 1.8 per cent. both before and after excitation, and he concluded that neither urea nor other nitrogenous compounds had anything to do with the production of electricity. Baglioni (1906*a*, 1917*b*) made analyses of water, solids, total nitrogen, protein, urea, glycogen, sodium and potassium of the muscle, electric organ and serum of the torpedo, and although he did not look for a change in urea content of the electric organ after excitation, he

comments on the great diffusibility of urea and its nearly uniform distribution throughout the body.

Buijtendijk (1909*a*) showed that, after bleeding, the blood is replaced by a solution richer in salt and poorer in urea. Denis (1913) made analyses of non-protein nitrogen, ammonia, uric acid, creatine and creatinine in the serum of *Mustelus canis*, *Carcharias littoralis* and *Raja erinacea*, as well as similar analyses on the blood of a number of teleosts, and in a later paper (1922) she reports some re-analyses of teleost blood, and gives additional figures on *Mustelus canis*, *Carcharias* and *C. littoralis*. Further data on the blood of the skates, the conger eel and several mammals are given by Delaunay (1913).

The most extensive series of observations on different species of Elasmobranchii is that presented by Kisch (1930). Using a xanthidrol gravimetric method, Kisch found the urea content of the blood to vary in *Torpedo ocellata* from 1.55 to 2.0 per cent.; in *T. marmorata* from 1.45 to 1.84 per cent.; in *Scyllium canicula* from 2.08 to 2.64 per cent.; and in *Mustelus laevis* from 1.5 to 2.1 per cent. Kisch's work was done in Naples where one may safely presume a uniformity of environment, and although there was no marked difference between the sexes, his figures leave no doubt that there may be a marked variation in normal animals of the same species. The average blood urea for various species also shows striking differences: *Torpedo ocellata* 1.72 per cent., *T. marmorata* 1.63 per cent., *Scyllium canicula* 2.35 per cent., *S. stellare* 2.40 per cent., *Raja asterias* 2.40 per cent., *Trygon pastinaca* 1.36 per cent. (one specimen only), *T. violacea* 2.25 per cent., *Squatina angelus* 2.48 per cent., *Sphyrna zygaena* 1.85 per cent., *Mustelus laevis* 1.79 per cent. Kisch observed that, though the blood urea usually falls in fasting, in some instances it rises by a considerable amount instead, and while haemorrhage, asphyxia and removal of the liver have no consistent effect, the level of blood urea usually falls in moribund animals. Urea is present in slightly varying ratios to the blood in the aqueous humour, perilymph, endolymph, cerebrospinal, pericardial and perivisceral fluids, gall and bile. Incidentally, it may be noted that according to Kisch's figures the electric organ has only 11 per cent. solids in contrast to 22-25 per cent. in the muscle, a fact that may have a bearing on the relative richness of this organ in urea, as observed by Krukenberg and by Gréhan and Jolyet.

The urea content of the plasma, cerebrospinal, perivisceral and pericardial fluids of several species of elasmobranchs determined by urease decomposition and Nesslerisation, has been reported by the author (Smith, 1929*a*). Macallum (1926) found large quantities of ammonia in dogfish serum. We were unable to confirm this fact and have suggested that in the sample Macallum analysed ammonia had been formed by decomposition. The pericardial and perivisceral fluids may contain as much urea as the serum, but in some instances the concentration is considerably less. This unequal distribution is significant in view of the approximately equal distribution of this substance per kg. of water between the serum and tissues, as was shown by von Schroeder, and confirmed by us, and it becomes all the more significant when it is recognised that, in regard to inorganic composition, these fluids are highly differentiated from the plasma.

A sufficiently large number of fresh-water teleosts, marine teleosts and terrestrial animals have been examined (Krukenberg, 1881; Schöndorff, 1899; Buglia and Constantino, 1912; Denis, 1913, 1922; Wilson and Adolph, 1917; Scheunert and Pelchrzim, 1923; Schulz and Krüger, 1925; Fearon, 1926) to preclude reasonably the possibility of a physiological uraemia, similar to that observed in the Elasmobranchii, occurring elsewhere in the animal kingdom. There is no evidence to controvert von Schroeder's view that the urea is freely diffusible within the organism, permeating the blood and tissues in proportion to the water content. Krukenberg's thesis of a special affinity for urea on the part of tissues seems to be unwarranted, although it may prove to be true that certain membranes (*i.e.* the péricardial and perivisceral membranes) possess to some special degree the power of restraining its diffusion. So there remain the problems of discovering the function of the urea in the organism, and of examining the physiological mechanisms by which the uraemic state is brought about.

III. THE OSMOTIC PRESSURE OF ELASMOBRANCH BLOOD.

Claude Bernard's thesis of the constancy of the *milieu intérieur*, in contrast to the variability of the *milieu extérieur*, as an essential condition for the maintenance of life in the higher animals, dates from 1859. It was soon recognised that the salt composition of the blood was one essential feature in which this constancy of composition was exemplified, and the early experiments of Frédéricq were directed towards an analysis of the properties of this fluid in aquatic animals, in relation to those of the surrounding medium.

Concurrently, it came to be recognised from the work of Arrhenius, Van't Hoff, Pfeffer and others that the osmotic pressure of a salt solution represented an essential feature governing the movement of water across semi-permeable membranes, and biologists began to think of the gills and integumentary membranes of aquatic animals as analogous to, if not identical with, the semi-permeable membranes upon which the physical chemists had erected the foundations of osmotic theory. It was to be expected that biologists, with Bernard's thesis in mind, would turn immediately to the marine and fresh-water fishes where problems concerning the distribution of salt and water between blood and environment were presented in their most obvious form.

The cryoscope was introduced into biology towards the close of the century, and Bottazzi was the first to apply it to the measurement of osmotic pressure of fish blood in 1897. Bottazzi found the blood of the elasmobranchs to be about isotonic with sea water (in this respect resembling the marine invertebrates), whereas the blood of teleosts had a much lower osmotic pressure, and one that tended to remain constant whether they lived in salt or fresh water. He suggested that in the teleosts there was evident for the first time an independence of the organism, whereby it could establish and maintain an osmotic pressure that was not influenced by changes in its environment. Though Bottazzi's conclusions need extensive qualifications, they represent the first significant synthesis in this problem.

Bottazzi's description of the blood of elasmobranchs as isotonic with sea water was in contradiction to Frédéricq's observation that elasmobranch blood contains considerably less salt than does the sea, and it remained for Rodier (1899 and 1900) to resolve the contradiction by pointing out that it was the urea, which Städelér and Frérichs had discovered, that accounted for the difference. Rodier showed that the blood serum, the pericardial, perivisceral and uterine fluids are essentially isotonic with each other, and he called attention to one point that has since proved to be very significant: contrary to Bottazzi's description, in many instances the osmotic pressure of elasmobranch blood was distinctly greater than that of the sea water from which the animal had been removed. He was unable, however, to decide whether this was a permanent condition or a consequence of incomplete adaptation. Quinton (1897, 1900) had suggested that in its composition the blood is but diluted sea water; but Rodier, by analyses of chlorine and magnesium, demonstrated that the several body fluids differed significantly in the relative proportions of these salts, from each other and from sea water, and he recognised that differences in the composition of the water to which the animals were acclimatised might be responsible for differences in the composition of the blood.

In line with his previous observations on the salt content of the blood of various animals in relation to changed environment, Frédéricq (1904) undertook to determine the nature of the bounding membranes by which the blood is isolated from the exterior. He distinguished three steps or degrees of physiological regulation; one, in which the internal environment presents the same molecular composition and osmotic pressure as sea water, as represented by the marine invertebrates; in the second class the internal environment has the same osmotic pressure as the water in which the animal lives, but the salt content is very much less, the difference in osmotic pressure being accounted for by the presence of organic substances, this class being represented by the elasmobranch fishes; in the third class both the osmotic pressure and salt content of the blood are very different from the environment, and in this class he included the marine and fresh-water teleosts and certain fresh-water invertebrates. He recognised that equality between blood and sea water in the elasmobranchs was not reached by diffusion of osmotically active substances, since the highly diffusible urea is held within the body and salts are held outside. And from experiments with nitrate and with diluted and concentrated sea water he concluded that the gills are in truth impermeable to salts as well as urea, osmotic equilibrium across them being attained by the passage of water only. He therefore considered the gills as highly semi-permeable and looked upon the development of this semi-permeability as a step by which the organism perfected its isolation from the external world. He thought that a repetition of the evolutionary sequence exemplified by the invertebrates and lower vertebrates could also be discerned in the physiological isolation of the tissues from the body fluids. There is little in Frédéricq's views that does not require extensive revision in the light of later work, yet his theory of a progressive evolution of body fluid regulation is historically important.

Bottazzi (1906*a, b*) showed that elasmobranch urine is approximately isotonic

with the blood, as is also the bile, and he was the first to observe that the blood cells haemolyse in urea solutions having the same osmotic pressure as the blood and appear to be freely permeable to this substance (1906*c*), though Rodier (1899) had suggested this, Frédéricq (1901*b*) had clearly believed it, and Dekhuyzen (1904) had asserted it as a fact. Bottazzi's observations on the osmotic pressure of elasmobranch blood and body fluids of many animals are summarised in his review of 1908. Schmidt-Nielsen and Schmidt-Nielsen (1923) have shown that the blood of *Chimaera monstrosa* has about the same osmotic pressure as sea water, and Dakin (1931) has found the same thing for *Callorhynchus millii*, thus bringing the Chimaeroidei into line with the other Elasmobranchii. (See also Dakin, 1935.)

Scott and Denis (1913) attempted to examine the permeability of the gills of the dogfish by diffusion experiments with animals in which the cord had been sectioned and the oesophagus obstructed by a bolus of oiled cotton. They added potassium iodide, boric acid, and methylene blue to sea water passing through the branchial cavity and were able to demonstrate the presence of these substances in the blood and urine, and they concluded that the gills were "permeable" to them. Their experiments, however, are scarcely convincing.

The most careful and extensive examination that has been made on the osmotic pressure of elasmobranch blood are the observations of Duval and Portier (1923) and the subsequent investigations of Duval (1923*a-25a*). This work has been well summarised in the last reference, and only certain points need to be reiterated here. After carefully considering the work that had been done by previous investigators, Duval and Portier undertook to determine with greater accuracy the osmotic pressure of elasmobranch blood (by the freezing-point method) under a variety of conditions. They worked with *Trygon vulgaris*, *Scyllium canicula*, *S. catulus*, *Centrina* sp., *Galeus canis*, and *Raja undulata*. They point out a fact that Rodier had noticed, and which was evident in the data given by numerous other investigators, that the osmotic pressure of elasmobranch blood is frequently greater than that of the sea water from which they are removed. After a series of observations on animals that had been given time to acclimatise themselves to the sea water of the laboratory, they conclude that this *hypertonicity* of the blood is the normal state of affairs. This observation and conclusion have been confirmed by the author (Smith, 1931*b*). In having a blood that is hypertonic to the environment, the elasmobranchs are not only distinguished from the bony fishes but also from the majority of marine invertebrates. (It has since been established that a few marine invertebrates show a similar relation (Schlieper, 1930).) The difference in freezing-point between blood and sea water in Duval and Portier's data varies from 0.04 to 0.12° C. and averages 0.08° C., and although they emphasise this difference, they do not attempt to interpret it. Duval (1925*d*) estimates that, on the average, urea is responsible for 44 per cent. of the total osmotic pressure, and is therefore in large part responsible for the maintenance of the hypertonicity of the blood.

Overlooking Bottazzi's observations on the point, Duval believed that he was the first to show that the red blood cell of the elasmobranch is permeable to urea. He demonstrated this by measuring the red cell volume by the haematocrit method in

solutions containing varying proportions of sodium chloride and urea. The fact of this permeability led him to distinguish the "physiological osmotic pressure" of the serum with regard to the tissues, from "physical-chemical osmotic pressure" as measured by the freezing-point method; but he accepted in principle Frédéricq's contention that the urea is osmotically important with regard to the external environment, because of the fact that the gills and the integumentary membranes are impermeable to this substance. He demonstrated this latter fact by keeping the animals in mixtures of sodium chloride and urea. When placed in dilute sodium chloride solutions the freezing-point of the blood changed in a characteristic manner, and the substitution of urea for sodium chloride was effective in preventing this change, thus showing that the urea does not diffuse from the exterior to the interior; and as he points out, the fact of the accumulation of the urea in the animal indicates that it does not diffuse (freely, at least) from the interior to the exterior.

IV. THE CARDIAC THEORY OF THE FUNCTION OF UREA.

In 1901 Straub, during a pharmacological examination of the action of digitalis and strophantin on the elasmobranch heart, found that he could not keep this organ in good condition in 3.4 per cent. sodium chloride, a solution which had the same total osmotic pressure as the blood. Starting from this observation, Baglioni (1905) examined the effects of various sodium chloride solutions upon the excised heart and came to the conclusion that urea is absolutely necessary for cardiac activity, and, by inference, for the activity of all other tissues of the Elasmobranchii. The heart would beat satisfactorily, he said, only in a solution containing 2 per cent. sodium chloride and 2.2 per cent. urea.

The next year (1906*b*) he collected urine in the dogfish and discovered that the kidneys do not tend to excrete the whole quantity of urea that is present in the blood, as is the case in the higher animals, but only a small part of it, so that the blood always maintains the same chemical composition. This seemed natural enough when the urea was viewed as a substance indispensable to life, and Baglioni pointed to the retention of urea by the elasmobranch kidney as analogous to the retention of sodium chloride and other substances in the higher vertebrates. The urea functioned, he thought, by neutralising the harmful effects of the large quantities of sodium chloride in the blood.

There were, in the current literature, numerous observations that tended to support Baglioni's point of view. It had been shown that small amounts of urea, when added to saline perfusion fluids, altered the tone of smooth muscle and promoted the activity of cardiac muscle in the frog, rabbit, etc. But Bottazzi (1906*c*) criticised Baglioni's experiments on the ground that the red blood cells of the shark haemolysed in a urea solution isotonic with the plasma, indicating that they were permeable to this substance; he referred to and confirmed Rodier's (1899) observation that the cells of several elasmobranch species were isotonic with 1.3-1.6 per cent. sodium chloride, and he argued, therefore, that a 3.4 per cent. sodium chloride solution was probably hypertonic for the elasmobranch heart, if the heart were equally permeable to urea.

Baglioni (1907*a, b*) answered that 2 per cent. sodium chloride failed to keep the heart in good condition, and he went further by trying various mixtures of urea and sodium chloride, and of sucrose and sodium chloride, all having the same freezing-point (-2.0°C.) as elasmobranch blood, and found that the one that worked best was the mixture containing 2 per cent. sodium chloride and 2.2 per cent. urea. In urea solutions the tone of the heart muscle was increased and contraction was arrested in systole; in sodium chloride solutions, on the contrary, the tone of the heart muscle was decreased and contraction was arrested in diastole. So he concluded that the two substances were mutually antagonistic, and that the normal contractility was dependent upon their mutual interaction.

Fühner (1908) reported experiments with excised heart muscle in which he used Baglioni's fluid, and De Meyer (1910) studied the effect of pure sodium chloride or pure urea solutions upon the action currents accompanying contraction. The latter concluded that there was an intimate relationship between the effect of oxygen and the effect of urea on contractility, but in retrospect his experiments appear to be open to other interpretations. Mines (1912) inferred that the effect of the removal of urea is in part due to the change in osmotic pressure of the fluid, as is shown by the action of non-electrolytes such as cane-sugar or glucose. When these are substituted in equal molecular proportions for the urea, there is no immediate falling off of the beat; indeed with cane-sugar there is some increase, but after a few minutes an irregularity sets in and the beats become weakened, to be restored by return to a medium containing urea. Though he failed to devise a urea-free fluid that was entirely satisfactory, he quotes a personal communication from Knowlton to the effect that it was possible to prepare an alkaline perfusion fluid containing dextrose but no urea, which would keep the dogfish heart beating satisfactorily. He could detect no utilisation of urea in a solution in which hearts had been left beating for 20 hours, and he concluded that the general relations of the elasmobranch heart to electrolytes were so similar to those of the frog's heart that it seemed probable that the existence of this special chemical condition—the need for the presence of urea—denoted no very far-reaching difference in the cardiac mechanism.

Bompiani (1913) examined the effects upon the heart of replacing the urea in the perfusion fluid by methyl-urea, ethyl-urea, phenyl-urea, sulphur-urea, guanidin, biuret, alloxan, ammonium carbonate, glycerine, acetone, urethane, glycol and asparagin. None of these worked as well as urea, though methyl-urea worked nearly as well, maintaining a heart for four-fifths of the time that a control preparation survived in Baglioni's solution. He concluded the action of urea involved some specific chemical property.

In 1917*b* Baglioni, reviewing his position, reasserted his original thesis that urea was necessary for the maintenance of physiological activity in the heart and other tissues of the elasmobranch, and placed this substance in the class of chemical compounds which Bayliss and Starling had called hormones. Pointing to the role of the waste product, carbon dioxide, in the regulation of respiration and other physiological processes, he asserted that there was no difficulty in believing that urea could exert an excitatory action or have other physiological attributes, not only in the

elasmobranch but also in the teleosts, Amphibia and mammals. The fact that pure urea solutions caused systolic arrest in the elasmobranch heart led him to postulate an excitatory action on the part of urea, and he extended this theory to explain the elevated blood pressure as well as the hypertrophy of the heart in chronic nephritis in man. It was even possible, he thought, that phenomena of central excitation observed in nephritis might be of strictly "uraemic" origin.

The only observer who has attempted to controvert Baglioni's experiments is Frédéricq (1922), who has confirmed the fact that a 3.5 per cent. sodium chloride solution (which is isotonic with the blood) is incapable of maintaining contractility in the *Scyllium* heart, whereas a solution containing 2 per cent. sodium chloride and 2 per cent. urea is satisfactory for this purpose. Frédéricq, however, repeated Baglioni's experiments with weaker salt solutions and found that the heart of the dogfish would continue to beat for a long time when constantly washed with a fluid that is entirely urea-free. It is sufficient that this fluid have approximately the same sodium chloride concentration as the serum, and that it contain sodium, potassium and calcium in definite proportions. Urea plays an important role, Frédéricq concluded, in regard to the osmotic pressure of the blood as opposed to the osmotic pressure of the sea water which bathes the branchial lamellae, but it is not important osmotically or chemically with regard to the tissues, which are freely permeated by it. The subject recently has been examined by Simpson and Ogden (1932) who, disregarding investigations previous to Mines', found that the heart would not maintain its contractility in solutions in which sucrose, sulphate or thio-urea had replaced the urea in equal molecular quantities.

Baglioni's interesting theory of the physiological action of urea rests solely upon his experiments with the elasmobranch heart. These were performed at a time when the composition of satisfactory perfusion fluids was even much more obscure than it is now. The problem of maintaining excitability and contractility in excised cardiac muscle is an extraordinarily complex one, involving not only an appropriate balance of electrolytes and a suitable osmotic pressure, but quite possibly optimum concentrations of relatively inactive chemical substances such as proteins, amino acids or lipoids, some of which substances function physically, or indirectly through combination with calcium and other ions. Urea, for all the evidence to the contrary, may have some such action not only in the elasmobranch heart but also in the heart and tissues of other animals. This would not imply, however, a specific function such as Baglioni hypothesized. A negative experiment in this problem is scarcely significant; it is much more significant that Frédéricq, using a balanced salt solution, was able to obtain quite satisfactory results with a urea-free solution. In no case would it be expected, however indifferent urea might be, that one could abruptly transport heart muscle from a serum containing 2 per cent. of this substance to a salt solution containing none of it without some disturbance. I am inclined, therefore, to discount Baglioni's results and, since none of the subsequent investigators has done other than accept his results uncritically, to conclude from general considerations and from Frédéricq's experiments that his theory is unproved. Frédéricq's hypothesis is much more attractive, namely, that the urea functions in an

osmotic role across the branchial lamellae, which are impermeable to it, while within the organism it is essentially inert.

V. EFFECTS OF THE TRANSFER TO DILUTED OR CONCENTRATED SEA WATER.

In view of the fact that many fishes migrate from fresh water to the sea or in the reverse direction, and since some of these, at least, tolerate abrupt transfer from one medium to the other, investigators have frequently attempted by this method to unravel the complex physiology of osmotic regulation. The knowledge that has been gained by such experiments is scarcely worth the effort that has been put into them, for it has been found that in general fishes acclimatised to either salt or fresh water die very shortly after transfer to the other medium, and consequently all results obtained by such a procedure must be qualified by the knowledge that the animal is, to say the least, handicapped by being presented with very abnormal conditions. The extent of the error into which such transfer experiments can lead is illustrated by the fact that most observers have concluded from them that the elasmobranch fishes will not live in fresh water, whereas, as is shown in Section VII, a great many genera, not to say species, can tolerate fresh water for an indefinite period of time, and many of them breed in fresh water and probably are permanently established there. Transfer experiments are interesting, nevertheless, for having revealed the physiological limitations of the organism in meeting the new conditions.

Mosso (1890-1) put sharks into fresh water and concluded that death was in part due to branchial congestion which in turn led to suffocation. Later, with the question of the permeability of the branchial membranes to salt and water in mind, Frédéricq (1901*a*, *b*; 1904) transferred dogfish to diluted sea water or to sea water that had been enriched with sodium chloride; he observed that the osmotic pressure of the blood changed under these conditions, and since he was unable to demonstrate the penetration of nitrate, he concluded that osmotic equilibrium was maintained by the transport of water, which he considered moved through the gills as through any semi-permeable membrane. He believed that the blood maintained the same osmotic pressure as that of the external medium under all conditions (see also Quinton, 1904). Garrey (1905) pointed out that the dogfish did not endure diluted sea water well and that true osmotic equilibrium was probably never reached under the conditions of such experiments.

Scott and White (1910), by measurements of plasma protein and chloride, observed that the blood was diluted in a dogfish immersed in fresh water but, because some chloride escaped from the gills, they concluded that these were permeable to salts. Subsequently Scott (1913, 1916) pointed out that the extent to which the blood changes its composition varied considerably with the conditions of the experiment and with the time of immersion in the new medium, and that the more advanced changes are not reversible if the animal is returned again to sea water. He believed that the water exchange was effected primarily through the gill membranes, but that a diffusion of salts also occurred. He adduced evidence that the kidneys operate to maintain the normal osmotic pressure of the blood by excreting

an increased volume of diluted urine, conserving salts and urea; yet ultimately respiration and circulation are affected and death occurs in consequence of disturbances in these vital functions. Scott examined *Squalus* living in brackish water, and concluded that within limits the kidneys could compensate for the increased absorption of water, under which condition the animal succeeded in maintaining the osmotic pressure of its blood at a level considerably above that of the external medium.

Backman (1914) found that a short exposure to diluted sea water caused the oxygen tension of the blood to fall to low values independently of blood dilution, a result that he attributed to local congestion and swelling of the gills.

In Frédéricq's experiments only a moderate change in environmental osmotic pressure was attempted, so that some of his animals survived 24 hours, whereas in the other investigations noted the experimenters were content if the animal survived from 1 to 6 hours. Duval (1925*b*) recognised the significance of this point and attempted to arrange his experiments in such a manner that the animals would survive for as long a time as possible. He was not successful in keeping the animals alive for long, however, and admitted that often as not the fish were dying at the conclusion of the experiment. Recognising that osmotic equilibrium was never reached between the blood and the mixture in which the animal was placed, his results, however, seemed to warrant the conclusion that in diluted sea water the elasmobranch did not maintain isotonicity, as had been thought by Frédéricq, Quinton and others, but tended to maintain the osmotic pressure of the blood at a level significantly above that of the external medium. Thus the state of hypertonicity characterising the elasmobranch in sea water was maintained in diluted sea water, the difference in osmotic pressure becoming progressively greater, the greater the dilution of the latter.

Similar transfer experiments have been performed by Quigley (1928) who also studied the effect of various salts and *pH*, and by Chaisson (1930) who believed that a difference in response to diluted sea water on the part of normal animals, as compared with animals in which the cord had been destroyed, indicated a participation of the central nervous system in osmotic regulation. Death in diluted sea water, Chaisson concluded, was due to injury of the gills or the medulla.

Margaria (1931) brought to the problem the vapour-pressure method of measuring osmotic pressure, but apart from observing an increase in weight of the animal, a dilution of the blood, and other consequences of abrupt transfer to fresh water, he added nothing to the problem except the erroneous deductions that the elasmobranch cannot maintain a difference of osmotic pressure between the blood and its environment, that the gill behaves as though it were an ideally semi-permeable membrane, that the kidneys are incapable of excreting excess water, and that the urine is fixed in amount and osmotic pressure. Hukuda (1932) has also concluded from short-time transfer experiments that the gills behave like a semi-permeable membrane. Further transfer experiments have been reported recently by Rowinski (1934).

It seems evident that the type of experiment cited above is not such as to reveal

either the physiological capacities of the elasmobranch to compensate for a change in the osmotic pressure of its environment, or the mechanisms by which this compensation might be effected. The animals in these experiments were obviously abnormal and taxed beyond their strength and, excluding Duval's work, the observations made upon them have led for the most part only to incorrect conclusions.

VI. THE EXCRETION OF URINE AND THE ROLE OF THE KIDNEYS IN UREA CONSERVATION.

Herter (1891) was the first to collect elasmobranch urine (*Scyllium* and *Torpedo*), but he made no analysis for urea. Baglioni (1906*b*), using the retention catheter method devised by Herter, added to the former's inorganic analyses data on urea excretion and the rate of urine formation. He found that the urea content of the urine rarely rose above one-third of that of the blood and that, after bleeding, the animal apparently retained nitrogen in order to restore the nitrogen content of the plasma. Burian (1908-9) first showed that elasmobranch urine was isotonic with, or in some instances hypotonic to, the blood—a point of the utmost physiological significance. (This is also true, as was shown by Burian and confirmed by many subsequent observers, of teleost urine. See Smith, 1930*a*, 1932.)

Buijtendijk (1909*b*) found that the osmotic pressure of the urine of *Scyllium canicula*, as well as the urea content, tended to rise during diuresis toward the level of the plasma. He observed that if he destroyed the dorsal half of the medulla somewhat below the fourth ventricle, he obtained a marked rise in the urea content of the urine so that the latter in some instances almost attained the plasma level.

Denis (1912) made further analyses of nitrogenous constituents in the urine of *Mustelus canis* and confirmed Baglioni in the observation that the urea content is invariably less than that of the plasma. For some inexplicable reason she failed to find creatine, although this substance is now known to constitute one of the principal nitrogenous constituents in the urine of all fishes (Smith, 1929*b*, 1930*b*). White (1931) has demonstrated the preponderance of creatine over creatinine excretion in *Squalus suckleyi*, thus bringing it into line with other cold-blooded animals. In the fishes, as in the birds and reptiles, creatine excretion predominates over creatinine, the latter being present only in traces.

The peculiar nitrogenous composition of fish urine is explained in part by the fact that a considerable fraction of the waste nitrogen of teleosts is excreted by the gills instead of by the kidney. The branchial excretion consists largely, if not entirely, of ammonia and urea, leaving creatine, creatinine, trimethylamine oxide, etc., predominating in the urine (Smith, 1929*b*).

Such information as is available on the distribution of nitrogen between urea and ammonia in the nitrogen metabolism of fishes indicates that ammonia may frequently, if not usually, be the predominant form. In studies of the lungfish (Smith, 1930*b*), however, it has been shown that whenever nitrogen accumulates in the body in consequence of conditions that do not permit its excretion, it accumulates not as ammonia, but as urea. During aestivation, for example, the degraded protein nitrogen is stored exclusively as urea, there being no accumulation of

ammonia in the body whatever. In the mammals urinary ammonia is apparently formed in the kidneys, there never being any large amount of this substance in the blood, and it seems likely that in fish the formation of ammonia is likewise a peripheral function, although here one must suppose that the gills as well as the kidneys participate in its elaboration. It is not known, in any case, whether the ammonia is made from urea or from some other nitrogenous precursor. Delaunay (1929*b*) has suggested that the teleosts excrete nitrogen primarily in the form of ammonia, but it would seem that this author did not take into account the fact that all animals that excrete nitrogen in the form of urea can, in accordance with varying physiological needs, deflect some of this nitrogen to ammonia, and that in the fishes the distribution between ammonia and urea is an extremely variable one.

In the studies of fresh-water elasmobranchs to be described in Section VIII I have demonstrated that a considerable part of the urea excreted by these animals is excreted through the gills, as is the case in the teleosts. When the total nitrogen excretion is referred to the simultaneous excretion of phosphate, the N : P ratio has a value to be expected from our general knowledge of the metabolism of proteins. In the absence of any evidence to the contrary, there is no reason to doubt that in the elasmobranchs generally, as in the teleosts, protein nitrogen is degraded to urea through the usual course of metabolism, except in so far as a variable fraction is deflected to and excreted as ammonia in relation to acid-base economy, or for other reasons.

The problem of accounting for the physiological uraemia of the elasmobranchs is reduced, then, to an examination of the means by which the urea formed from protein metabolism is retained in the body. In a previous section we have cited Duval's work showing that the skin and gills of these fishes are relatively impermeable to this substance. The one outlet remaining open is through the kidneys, and obviously we must look here for a physiological mechanism that will account not only for urea accumulation, but perhaps for a regulation of the quantity in the body, or, more particularly, of the plasma level.

Increasing knowledge of the physiology of the kidney makes it clear that urine formation in the elasmobranchs, as in all other vertebrates except the aglomerular fishes, is effected by an initial filtration of protein-free plasma at the glomerulus with a subsequent modification of the composition of this filtrate as it passes along the renal tubules. Both the reabsorption of such valuable constituents as are incidentally (and necessarily) present in the filtrate, and the secretion of waste products from the blood, play a part in the final elaboration of the urine by the tubules. Walker and Elsom (1930) have shown that in the frog, urea is filtered through the glomerular capillaries, and there is ample reason for believing that this must also be true of the elasmobranch. If it were not filtrable, the urea in a 2.5 per cent. solution would exert an osmotic pressure of over 7,000 mm. of mercury; even in the absence of exact knowledge on the glomerular blood pressure in the elasmobranch kidney, we may believe that if no more than 1 per cent. of this urea were not filtered it would, by its osmotic pressure, effectively block the filtration process completely. Marshall (1930) has shown that *Raja erinacea*, *Squalus acanthias* and

Mustelus canis excrete ferrocyanide, and it is known that *Squalus acanthias* excretes xylose, sucrose, inulin and—under the influence of phlorizin—glucose (Clarke and Smith, 1932; Shannon, 1934*a, b*); there are good reasons for believing that these substances are excreted only by glomerular filtration (Smith, 1935; Shannon and Smith, 1935), and with numerous active glomeruli it would be difficult to believe that urea was not also filtered in the elasmobranch kidney.

Accepting this highly probable supposition, it follows from the relatively lower concentration in the urine that the urea must be reabsorbed by the tubules. Since the reabsorption involves the movement of urea from a low concentration in the urine to a high concentration in the plasma, this reabsorptive process must be an active one in the same sense in which glucose is actively reabsorbed from the urine by the tubules of all glomerular vertebrates. Calculations based on the rate of glomerular filtration in the dogfish indicate that normally about 90 per cent. of the filtrated urea is reabsorbed (Clarke and Smith, 1932, 1936; see also Shannon, 1934*b*, for data on filtration rate).

It is interesting to note that the elasmobranch kidney is unique in having a tubular segment interposed between the glomerulus and the proximal segment that is not present in any other vertebrate (Borcea, 1906). It is possible that this segment is responsible for the reabsorption of the urea. (For literature on the fish kidney, see Marshall, 1934, 1935.)

VII. THE DISTRIBUTION OF FRESH-WATER ELASMOBRANCHS.

It is frequently stated that the elasmobranchs are exclusively marine forms, with the implication that they will not live in fresh water. For this reason I have included in Table I a list of fresh-water species. I am indebted to Mr Robert Matthews for assistance in the preparation of this list. With the exception of Engelhardt (1913) who listed twenty-two species in fresh water, such a tabulation has not, to my knowledge, been prepared before, and it is probable that the present one is far from complete. Some of the listed forms represent rare invasions into fresh water, but many species are permanently established there. *Ellipesus* and *Paratrygon* are stated to be exclusively fresh-water genera, and it is quite certain that some species of *Dasyatis*, *Potamotrygon*, *Raja* and *Carcharias*, as well as cosmopolitan *Pristis*, remain indefinitely in fresh water, breeding there and never necessarily returning to the sea, so that the distinction appears to be a nominal one.

The order Chimaeroidei, notable for being absent from the fresh-water list, has but one surviving family, the Chimaeridae, which is composed of three genera—*Chimaera*, *Callorhynchus* and *Harriotta*. With the probable exception of *Chimaera coliei*, these are typical abyssal forms and represent survivors of an elasmobranch stock that has probably been resident for a long time in deep water. This fact perhaps accounts for their absence from fresh water.

It is not clear why the equatorial rivers are invaded so much more readily by marine fish in general, as well as by the elasmobranchs, than are the rivers of the temperate zones. Tropical rivers frequently present a broad, slow-moving sweep

Table I. *Distribution of fresh-water Elasmobranchii.*

Genus	Species	Locality	Author
<i>Carcharhinus</i> (1)	<i>melanopterus</i> (Quoy and Gaimard)	Perak	Smith, 1931
"	<i>borneensis</i> (Bleeker) (7)	Baram	Fowler, 1905
"	<i>nicaraguensis</i> (Gill and Bransford)	Rio San Juan	Eigenmann, 1893
"	"	Lake Nicaragua	Meek, 1907
"	<i>gangeticus</i> (Müller and Henle)	Hoogly	Fowler, 1930
"	"	Baghdad	Day, 1889
"	sp. "	Calcutta	Hamilton, 1822
"	<i>zambesensis</i> (Peters)	Gambia	Svensson, 1933
"	"	Zambesi	Boulenger, 1909
<i>Hypoprion</i> (2)	<i>hemiodon</i> (Müller and Henle)	Calcutta	Day, 1889
<i>Sphyrna</i> (3)	<i>blochii</i> (Cuvier)	Tale Sap	Hora, 1924
"	<i>zygaena</i> (Linnaeus)	Turner's Bay	Fowler, 1933
<i>Scoliodon</i>	<i>walbeehmi</i> (Bleeker)	Tale Sap	Hora, 1924
"	<i>palasorrah</i> (Cuvier) (8)	Baram	Fowler, 1905
<i>Mustelus</i>	<i>canis</i> (Mitchill)	La Plata	Eigenmann, 1909
"	"	Calcasieu	Fowler, 1933
<i>Squalus</i> (4)	<i>acanthias</i> Linnaeus (9)	Scandinavia	Feddersen, 1880
<i>Pristis</i>	<i>microdon</i> Latham (10)	Rio Chucunaque	Breder, 1927
"	"	Gresik	Bleeker, 1858
"	"	Surabaya	"
"	"	Perak	Smith, 1931
"	"	Amazon	Garman, 1913
"	"	Mahanadi	Day, 1889
"	"	Zambesi	Boulenger, 1909
"	"	Gambia	"
"	"	"	Svensson, 1933
"	"	Borneo	Weber, 1894
"	"	Sumatra	"
"	"	Manila	Meyer, 1876
"	"	San Juan	Eigenmann, 1920
"	"	East Indies	Weber, 1894
"	<i>xyson</i> Bleeker	New South Wales	Whitley, 1927
"	"	Lynd	Whitley, 1928
"	"	Bandjermassing	Bleeker, 1852
"	"	Gambia	Budgett, 1899
"	"	Baram	Fowler, 1905
"	<i>pectinatus</i> Latham	Atrato	Eigenmann, 1920
"	<i>cuspidatus</i> Latham	Not defined	Garman, 1913
"	"	China	Fowler, 1930
<i>Dasyatis</i> (5)	<i>akajei</i> (Müller and Henle)	Yangtze	Tchang, 1929
"	<i>zuei</i> (Müller and Henle)	"	"
"	<i>uarnak</i> (Forsk.) (11)	Perak	Smith, 1931
"	"	Pengaron	Bleeker, 1858
"	"	Bandjermassing	"
"	"	Yangtze	Tchang, 1929
"	"	East Indies	Weber, 1894

Genera: (1) *Carcharias*, *Eulamia*, *Priodon*; (2) *Carcharias*; (3) *Cestracion*, *Zygaena*; (4) *Acanthias*; (5) *Trygon*, *Hypolophus*, *Raja*; (6) *Trygon*, *Taenura*.

Species: (7) *tephrodes*; (8) *acutus*; (9) *vulgaris*; (10) *perroteti*; (11) *pareh*, *undulata*; (12) *brevicauda*; (13) *hum-bolii*; (14) *dumerilii*, *mülleri*; (15) *segratus*; (16) *thouamianus*; (17) *rostrata*.

Among the other Selachii discussed in various papers in the bibliography the following synonyms are frequent (the first being the recommended form). To prevent confusion the author's usage has been followed in the text. *Scylliorhinus caniculus* (L.) = *Scyllium canicula*, *S. catulus*; *Scylliorhinus stellaris* (L.) = *Scyllium stellare*; *Odontaspis littoralis* (M.) = *Carcharias littoralis*, *C. canis*; *Mustelus mustelus* (L.) = *M. laevis*, *M. vulgaris*; *Mustelus canis* = *Galeus canis*; *Squalus acanthias* L. = *Spinax acanthias*, *Acanthias vulgaris*; *Squalus suckleyi* (G.) = *S. sucklii*; *Squatina squatina* (L.) = *S. angelus*; *Raja batis* L. = *R. undulata*; *Torpedo marmorata* R. = *T. galvani*; *Torpedo torpedo* (L.) = *T. ocellata*; *Dasyatis pastinaca* (L.) = *Trygon vulgaris*; *Dasyatis violacea* (B.) = *T. violacea*; *Hydrolagus coliei* (L. and B.) = *Chimaera coliei*.

The author is indebted to Mr J. T. Nichols, Curator of Recent Fishes, Department of Ichthyology, American Museum of Natural History, for a careful analysis of the synonymy of the selachians referred to here, and for supplying the authoritative usage, based upon Garman's *Plagiostoma* (1913) and Jordan and Evermann's *Check-list of Fishes of North and Middle America* (1930).

(Table I continued on p. 65.)

Table I (cont.).

Genus	Species	Locality	Author
<i>Dasyatis</i>	<i>fluviatorum</i> Ogilby	Brisbane	Garman, 1913
"	<i>sabineus</i> Lesueur	Tropical America	"
"	"	Florida	Boulenger "
"	<i>imbricata</i> (Schneider) (12)	Baram	Fowler, 1905
"	<i>fluviatilis</i> (Hamilton-Buchanan)	Ganges	Chandhuri, 1912
"	"	"	Hamilton, 1822
"	<i>marginatus</i> (Blyth)	Calcutta	Day, 1889
"	<i>bleekeri</i> (Blyth)*	Tale Sap	Hora, 1924
"	<i>sephen</i> (Forskal)*	Java	Weber, 1894
"	"	Tale Sap	Hora, 1924
"	"	Ganges	Chandhuri, 1912
"	"	Baram	Fowler, 1905
"	"	Tale Sap	Smith, 1931
"	"	East Indies	Bleeker, 1858
<i>Potamotrygon</i> (6)	<i>brachyurus</i> Günther	British Guiana	Eigenmann, 1892, 1909, 1920
"	<i>magdalenae</i> Steindachner	Colombia	Eigenmann, 1920
"	"	Rio Branco	Eigenmann, 1892, 1909, 1920
"	<i>hystrix</i> Müller and Henle (13)	British Guiana	Eigenmann, 1892, 1909, 1912
"	<i>d'orbignyi</i> Castelnau	"	Eigenmann, 1892, 1907, 1909
"	<i>motoro</i> Müller and Henle (14)	"	Eigenmann, 1892, 1909, 1912
"	"	Amazon	Vaillant, 1879
"	<i>reticulatus</i> (Günther)	South America	Eigenmann, 1892, 1909
"	<i>orbicularis</i> (Block and Schneider)	Amazon	Vaillant, 1879
"	<i>laticeps</i> Garman	Obidos	Garman, 1913
"	<i>scobina</i> Garman	Cameta	" "
"	<i>circularis</i> Garman	Teffe	" "
"	<i>humerosus</i> Garman	Montalegre	" "
"	<i>signatus</i> Garman (15)	Paranahyba	" "
<i>Paratrygon</i>	<i>strongylopterus</i> (Schomburgk)	South America	Eigenmann, 1892, 1909
<i>Ellipenesurus</i>	<i>spinicauda</i> Schomburgk	Orinoco	Eigenmann, 1912
"	"	Rio Branco	Eigenmann, 1892
<i>Narcine</i>	<i>brasiliensis</i> (Olfers)	South America	Eigenmann, 1909
<i>Disceus</i>	<i>thayeri</i> Garman	Amazon	Garman, 1913
<i>Rhinobatos</i>	<i>thouini</i> Müller and Henle (16)	Baram	Fowler, 1905
"	"	Tale Sap	Hora, 1924
<i>Myliobatis</i>	<i>aquila</i> (Linnaeus)	La Plata	Eigenmann, 1909
"	<i>goodei</i> Garman	Rio Grande de Sul	Garman, 1913
<i>Rhinoptera</i>	<i>javanica</i> Müller and Henle	Tale Sap	Hora, 1924
<i>Stoasodon</i>	<i>narinari</i> (Euphrasen)	"	"
<i>Pteroplatea</i>	<i>micrura</i> (Schneider)	Baram	Fowler, 1905
<i>Raja</i>	<i>oxyrinchus</i> Linnaeus (17)	Ouse	Pascoe, 1883
"	<i>batis</i> Linnaeus	Yangtze	Tchang, 1929
"	<i>microps</i> Günther	La Plata	Eigenmann, 1909
"	<i>platana</i> Günther	"	"

approaching the sea with gradual declination and, as Engelhardt (1913) has pointed out, the change in temperature from sea to fresh water is not apt to be abrupt. Fresh-water invasion is largely restricted to 30° N. or S., a band wherein the mean yearly variation of water temperature does not exceed 5° C. It may be that a high calcium content favours the transition (see Breder, 1934).

It is clear, however, that we are not entitled to exclude the elasmobranchs from fresh water on physiological grounds. Given the proper ecological conditions, there is little doubt that most of the smaller forms, at least, could survive as well in fresh water as in the ocean.

* Professor Hugh M. Smith advises me that he has taken *D. bleekeri* and *D. sephen* in fresh water in the Menam River, Siam, in addition to the Tale Sap, as recorded by Hora.

VIII. UREA AND OSMOTIC PRESSURE IN THE FRESH-WATER ELASMOBRANCHS.

Through the favour of the John Simon Guggenheim Memorial Foundation I was permitted to examine certain fresh-water elasmobranchs in Siam and Malaya. Most of these observations were confined to *Pristis microdon*, though a number of analyses were made of the body fluids of *Dasyatis uarnak* and *Carcharhinus melanopterus* (Smith, 1931a). These analyses revealed that the osmotic pressure of the blood of the fresh-water forms is typically much lower ($\Delta = 1.0^{\circ}\text{C.}$) than in marine forms (at Salisbury Cove, $\Delta = 1.94^{\circ}\text{C.}$). This lower osmotic pressure is due to a reduction in both plasma urea and plasma chloride. We may take as typical values for the urea, 650 mg. per cent. for fresh-water elasmobranchs and 2100 mg. per cent. for marine elasmobranchs. Thus, the transition to fresh water is accompanied by a reduction of about 70 per cent. in the plasma urea, although the concentration of this substance remains much higher than is observed normally in the higher vertebrates (10–30 mg. per cent.). The chloride content of the blood of fresh-water elasmobranchs is decidedly lower than in the marine forms, typical values for the former being 170 mM. per litre and for the latter 230 mM. per litre. This represents a reduction of about 25 per cent.

Since the total osmotic pressure is reduced by about 50 per cent., the decrease in plasma urea (1600 mg. per cent.) accounts for about half of the reduction. This fact suggests at once that the urea is physiologically involved in osmotic adaptation, particularly in relation to a marine habitat. That the urea should persist in fresh water at a plasma level considerably above that of other vertebrates may be due simply to the continued operation of those physiological mechanisms that maintain the plasma urea in the marine habitat, for the fresh-water elasmobranchs are not primitively inhabitants of that medium, but have migrated there from the sea in relatively recent times.

The urine in fresh-water *Pristis* is hypotonic to the blood, all constituents being very dilute, notably the urea, which averages less than 100 mg. per cent. in contrast to 650 mg. per cent. in the blood. It is clear that renal conservation of urea is carried on in fresh water, as in the sea. Chloride and salts are present only in traces, and the freezing-point is only about -0.1°C. , in contrast to -1.0°C. for the plasma. The urine flow is large, ranging from 150 to 460 c.c., and averaging 250 c.c., per kg. per day. Data, taken from Smith (1931b), summarising the typical composition of blood and urine from elasmobranchs living in various habitats, are given in Tables II and III.

The greater part of the urea, ammonia and chloride excreted by fresh-water *Pristis* is excreted extrarenally. When the total urea + ammonia nitrogen is considered, the sum bears a probable ratio to the simultaneous phosphate excretion, indicating that urea and ammonia constitute the principal end-products of the degradation of protein nitrogen. That the extrarenal excretion of nitrogen is a normal process is indicated by the fact that the N : P ratios in the urine of freshly caught animals have excessively high values, indicating a deficiency of nitrogen.

Table II. Osmotic pressure of serum and urine in elasmobranchs in relation to habitat.

	Δ° C. water	Δ° C. serum	Δ° C. urine
<i>Raja stabuliformis</i>	1.860	1.983	1.685
<i>Raja stabuliformis</i>	1.850	1.929	1.915
<i>Raja erinacea</i>	1.850	1.922	1.920
<i>Raja erinacea</i>	1.850	1.922	1.892
<i>Raja</i> sp.	1.484	1.617	1.065
<i>Raja</i> sp.	1.484	1.607	0.895
<i>Raja</i> sp.	1.091	1.227	0.788
<i>Raja</i> sp.	1.091	1.366	0.536
<i>Pristis microdon</i>	0.0	1.02	0.10

Table III. Composition of elasmobranch serum in relation to habitat.

	Δ° C. water	Δ° C. serum	Cl mM. per litre	Urea mg. %
<i>Raja stabuliformis</i>	1.850	1.930	273	2010
<i>Raja diaphenes</i>	1.850	1.924	272	2143
<i>Raja</i> sp.	1.484	1.617	224	1855
<i>Squalus acanthias</i>	1.330	1.622	234	1490
<i>Raja</i> sp.	1.091	1.366	162	1250
<i>Pristis microdon</i>	0.0	1.02	170	780
<i>Dasyatis uarnak</i>	0.0	1.02	212	626
<i>Carcharhinus melanopterus</i>	0.0	0.90	158	618
<i>Hypolophus syphax</i>	0.0	—	146	489

The extrarenal excretion of urea appears to be a passive diffusion from the blood, since no marked variation in the rate of its excretion could be effected under a variety of experimental conditions. The extrarenal excretion of ammonia and chloride, however, appeared to be under physiological regulation, since the excretion of these substances could be dissociated from the excretion of urea and completely, or nearly completely, arrested.

We have cited the experiments of Duval and Portier (1923) as showing that the elasmobranch gill is impermeable to urea; these experiments consisted of immersing the fish in various urea-sodium chloride solutions and noting the change in osmotic pressure of the blood. Such experiments are no doubt adequate to demonstrate a low degree of impermeability, but we question that they do or can demonstrate absolute impermeability, and we doubt that such impermeability exists; it seems probable that the branchial epithelium must permit diffusion of urea to occur to some small degree when the concentration gradient is from 650 mg. per cent. in the blood to 0 mg. per cent. in the water.

It has been shown that most of the urea excreted by the teleosts escapes from the body by an extrarenal route, and we have given reasons to think that this route is the gills (Smith, 1929*b*). In the teleost the gills are apparently quite permeable to urea, and with the constant circulation of blood through the branchial capillaries, bearing 10–30 mg. per cent., it is not surprising that most of this should diffuse through to the respired water. The anatomical situation is, of course, precisely the same in the

elasmobranchs, and our observations have led us to conclude that they differ from the teleosts only in possessing branchial (and perhaps oral) membranes that are relatively less permeable to urea.

IX. THE REGULATION OF OSMOTIC PRESSURE IN FRESH-WATER AND MARINE ELASMOBRANCHS.

Before a final interpretation of urea retention in the elasmobranchs can be attempted, it is necessary to consider the broader question of the osmotic relations of the organism living in fresh as compared to salt water. Whether urea retention is viewed as an osmotic adaptation or not, it must be recognised that the mere presence of this substance in the marine elasmobranchs completely alters their osmotic relations as compared with the marine teleosts.

The organism living in fresh water is osmotically superior to the external medium (*i.e.* its body fluids have the higher osmotic pressure) and therefore it will invariably tend to absorb water (and to lose salt) spontaneously in consequence of the existing osmotic gradient. The organism living in salt water is osmotically inferior to its environment and tends, therefore, to lose water (and to absorb salt). In order to compensate for the inevitable changes in water and salt content of the blood and tissues, the fresh-water organism is required to excrete a fluid which is *hypotonic* to the blood; while the salt-water organism is required to excrete a fluid which is *hypertonic* to the blood. From an energy point of view there is little difference between these two processes, for both require that the organism do osmotic work by separating from the blood a salt solution of dissimilar osmotic pressure. From the physiological point of view, however, hypotonic and hypertonic excretion appear to be carried on quite independently.

For example, the capacity to excrete a *hypotonic* urine appears to have been evolved separately and independently of the capacity to excrete a *hypertonic* urine. The former is present in all vertebrates (Smith, 1932), while the latter is absent not only in the elasmobranchs (Bottazzi, 1906*a, b*; Buijtendijk, 1909*b*; Smith, 1931*b*), but also in the teleosts (Rodier, 1899; Dekhuyzen, 1905; Burian, 1910; Smith, 1930*a*), the Amphibia (Bottazzi, 1908; Brunacci, 1914, 1917; Przylecki, 1922), the Reptilia (Burian, 1910 and unpublished observations of our own), and only slightly developed, if present at all, in the birds (d'Errico, 1907); whereas it is well known that the mammals can excrete a urine that has several times the osmotic pressure of the blood. Thus the capacity to excrete a hypotonic urine appears to have been evolved very early in vertebrate history (before the elasmobranchs); it suffices to maintain the osmotic pressure of the blood in organisms which live in fresh water, or to whom fresh water is freely available. The capacity to excrete a hypertonic urine appears to have been evolved in mammals, or to a much smaller degree, perhaps, in birds. The hypertonic reabsorption of water is apparently effected in mammals by the thin segment of the loop of Henle (Peter, 1909; Starling and Verney, 1925; Crane, 1927), which first appears in birds but is completely evolved only in mammals; Peter has correlated the length of this segment in different mammals with the ability of the kidney to excrete a more or less concentrated urine.

In the absence of hypertonic urine excretion, the teleost is faced with the task of procuring water of an osmotic pressure not greater than the blood as a vehicle for carrying waste products out of the body; in fact, this water must be available in a hypotonic condition to begin with, in order to permit the addition of osmotically active waste products. In the marine teleosts this hypotonic fluid must be elaborated from a hypertonic environment, and in the face of a constant loss of water to that environment by osmotic leakage. This is accomplished by the ingestion of sea water and the branchial excretion of salts, leaving water available for the formation of the osmotically dilute urine (Smith, 1930a). The circumstances are such that the marine fishes must be constantly ingesting sea water, and constantly doing osmotic work at the gills in order to maintain a constant urine flow. Urine excretion is maintained, therefore, at unremitting physiological expense, and it is not surprising to find that the rate of urine formation in normal marine teleosts is very low, of the order of 3 c.c. per kg. per day (Grafflin, 1931; Clarke, 1934).

The fresh-water teleosts, on the other hand, not only have water *ad libitum*, but their major task is to get rid of the water that is constantly being absorbed. In these, the urine flow ranges from 100 to 300 c.c. per kg. per day (Marshall and Grafflin, 1932; Smith, 1930a).

The situation of the elasmobranch is very similar to that of the teleost, with this exception: the gills are relatively impermeable to urea, and the kidneys operate actively to store the substance in the body by reabsorbing it from the renal glomerular filtrate. As a diffusion process, the branchial excretion of urea is not amenable to physiological regulation, nor is it appreciably modified by moderate changes in the blood level of urea or by the osmotic or specific chemical equilibria of the organism. Since the urea is being produced at a constant rate by the metabolism of protein, it will tend to accumulate in the body more or less in inverse proportion to the rate of urine formation. Where water absorption is large, as in a brackish or fresh-water environment, the urine flow will be large and the urea will accumulate to only a moderate extent.

But upon entering salt water the organism finds itself in circumstances where water is no longer freely available to it, and the urine flow must decrease to low values and ultimately stop entirely except in so far as the branchial excretion of salts renders water available. The impermeability of the gills to urea, plus renal conservation and oliguria, leads to urea accumulation; this in turn raises the osmotic pressure of the blood until it rises above that of the external environment. The hypertonicity of the blood with respect to the sea water may be slight, as indeed it is according to the most reliable data, in all marine elasmobranchs, but it is none the less effective in completely reversing the osmotic gradient; no longer does water tend to be lost from the body to the environment by way of the gills and integument; on the contrary water tends to be absorbed to a slight extent (though not so much as in a fresh-water fish), so that urine formation is resumed at a low level, and urea is again excreted by the kidneys. This increase in urine flow tends to prevent further urea accumulation, and consequently the concentration of this substance in the blood tends to be maintained at a steady level.

Thus it is not going beyond a statement of the facts to visualise the two circumstances, branchial impermeability and renal conservation, as operating to

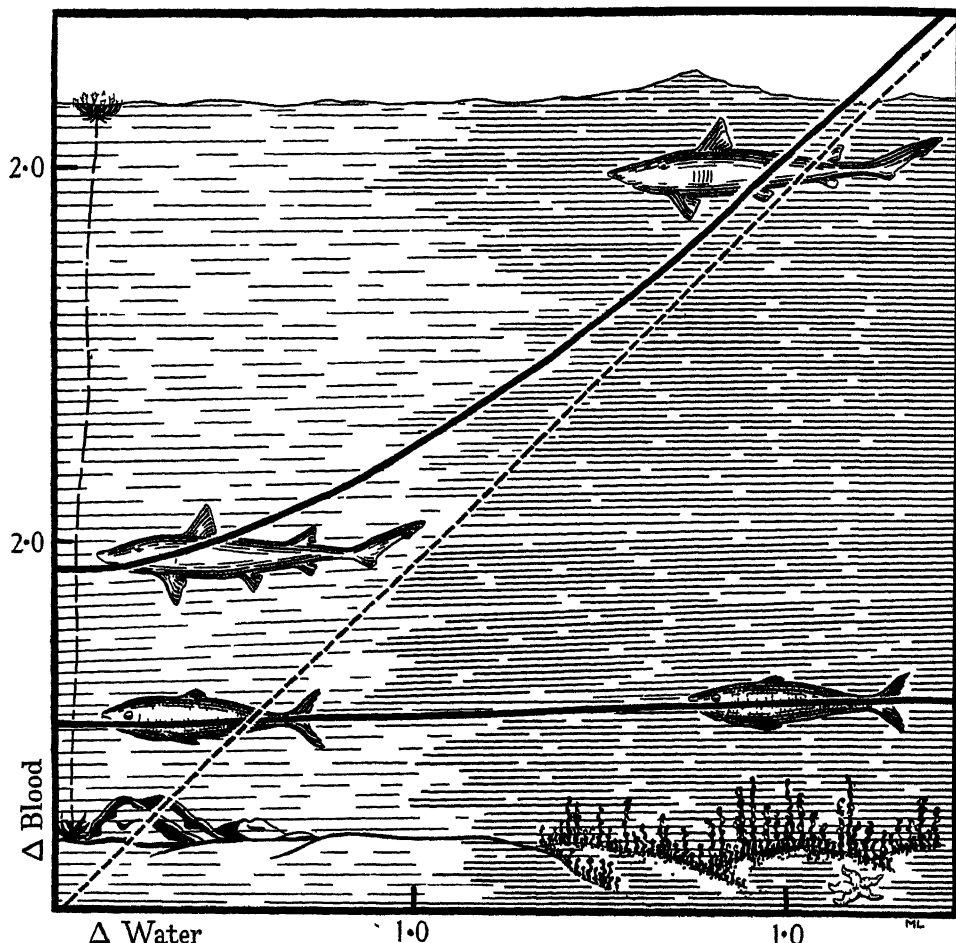


Fig. 1. In this figure the lowering of the freezing-point, Δ , is taken to represent the osmotic pressure of the environment on the one hand, and of the blood on the other. In fresh water, both the teleost and elasmobranch are osmotically superior to their environment; they therefore tend to absorb large quantities of water and have at all times adequate quantities available for the formation of hypotonic urine. In sea water the teleost is osmotically inferior to its environment and can obtain water for the formation of urine only at the expense of unremitting, extrarenal physiological labour (salt secretion). The elasmobranch, however, by virtue of its physiological uraemia, is osmotically superior to its environment both in fresh and salt water; although the margin favouring water absorption is small in the latter instance, the circumstances are such as to reverse completely the osmotic gradient and therefore to affect profoundly not only the maintenance of water equilibrium and the function of the kidneys, but also such remote features as the method of reproduction.

produce a low uraemic level in fresh water, and a high uraemic level in salt water, and to suppose that the uraemic level is conditioned primarily by the rate of urine formation, and therefore by the rate of water intake. The net result of this cycle is that the accumulation of urea automatically liberates the organism from osmotic

enslavement to a marine environment, and relieves it of the necessity of continuously doing osmotic work at the gills by hypertonic salt excretion, as is the case in the teleost.

The composition of the urine in marine elasmobranchs indicates that, like the teleosts, they excrete considerable quantities of sodium, potassium and chloride extrarenally, and one presumes that the branchial regulation of the osmotic pressure persists in some measure in them as a vestigial process. Perhaps the branchial operations represent the primitive mode of osmotic regulation—shared alike by the elasmobranchs and teleosts—upon which there has been superimposed the urea mechanism as a unique elasmobranch adaptation (Smith, 1931*b*).

In the light of the above interpretation it is not surprising that the abrupt transfer of elasmobranchs from salt to fresh water should be injurious. The animal is, so to speak, loaded with urea, and even under conditions of maximum urine flow a considerable period of time must elapse before that urea can be excreted from the body. Meanwhile, excessive quantities of water are absorbed, because of the excessive osmotic pressure of the blood, and the resulting hydraemia might well impair any physiological function.

The general osmotic relationships of the elasmobranchs and teleosts in fresh and salt water, as summarised above, are illustrated in Fig. 1.

X. THE RETENTION OF TRIMETHYLAMINE OXIDE.

In 1909 Suwa demonstrated that among the nitrogenous constituents of dogfish muscle, betain and trimethylamine oxide occurred in unusually large amounts. More recently, Hoppe-Seyler (1928–29, 1930) has shown that the latter substance is present in the blood in concentrations of 100–120 mM. per litre. Trimethylamine oxide is such a relatively weak base that its osmotic activity may be taken as roughly equal to its molecular concentration, and since the concentration of urea ranges from 330–440 mM. per litre, trimethylamine oxide appears to furnish 20–25 per cent. of the osmotic pressure referable to these two substances. There is no doubt that it must be considered with urea as a metabolite important in the osmotic regulation of these fishes. Its significance in this respect is emphasised by the fact that, as Hoppe-Seyler has shown, its concentration in the urine is 10 per cent. or less of the blood concentration. If it is filtrable at the glomerulus, these facts show that the elasmobranch kidney actively conserves it by tubular reabsorption, just as that organ conserves urea. And we may suppose that the branchial membranes are equally impermeable to it.

Hoppe-Seyler has shown that trimethylamine oxide is also present in small quantities in the blood and tissues of marine teleosts, and its presence has been demonstrated in the urine of *Lophius* (Grollman, 1929) and of *Myoxocephalus* (Grafflin and Gould, 1935), whereas it is apparently absent from fresh-water teleosts. Hoppe-Seyler considers that the retention of nitrogenous metabolites in the tissues and circulation plays an important part in bringing about the processes of methylation by which such compounds as betain and trimethylamine oxide are

found. If such is the case, the rapid excretion of nitrogenous waste products (due to the rapid urine flow) in fresh-water teleosts explains its absence in these forms.

Hoppe-Seyler has argued that the marine teleosts retain trimethylamine oxide for the same reason that the elasmobranchs do—to raise the osmotic pressure of the blood. But the validity of this argument appears open to question. Even if active nitrogen retention is assumed, it is apparent that it must be entirely ineffective in promotion of water absorption, for the quantity present is negligible in offsetting the osmotic deficit of the blood. But active retention can scarcely be assumed without proof of the fact; in view of the absence of active conservation of urea, and until evidence is adduced that the kidneys of marine teleosts do conserve trimethylamine oxide (*i.e.* that the blood normally contains more than urine), it seems more probable that the apparent retention of this substance by the marine teleosts is due simply to the facts that it is excreted exclusively in the urine (at least it does not diffuse through the gills) and that the rate of urine formation in these fishes is extremely small. In this view, a retention of urea would not be expected because it does diffuse so readily through the gills.

It is clear, however, that trimethylamine oxide must be taken into account in the elasmobranchs, not only in the problem of osmotic regulation and water equilibrium, but also in the problems centring around the physiology of the kidney.

XI. REPRODUCTION.

The majority of the Elasmobranchii are viviparous, the exceptions, so far as known, being the orders Chimaeroidei and Cestraciontes; and in the order Euselachii, the families Scylliorhinidae and Hemiscyllidae; in the order Tectospondyli, the family Somniosidae; and in the order Batoidei, the family Rajidae.

The order Cestraciontes includes among its living representatives the rare primitive sharks, *Chlamydoselachus* and *Heterodontus*. All the Euselachii are viviparous except the Scylliorhinidae and Hemiscyllidae, which include the common western dogfishes, *Scyllium canicula* and *S. catulus*, and the eastern *Chiloscyllium griseum* and *C. indicum*. The Greenland shark, *Laemargus borealis* (family Somniosidae), is unique in producing eggs devoid of a horny covering, which are deposited on the sea bottom (Jordan and Evermann, 1896).

All observers are agreed that oviparous reproduction is the primitive form, and that viviparous reproduction has been developed secondarily. In view of its biochemical significance the viviparous mode of reproduction is worthy of a brief description. In both oviparous and viviparous elasmobranchs fertilisation is effected by copulation of the sexes, and for this purpose the males are supplied with special copulatory organs called claspers. A large yolk-sac carries a supply of food for the embryo, in addition to that contained in the egg proper. In some viviparous species the horny egg-case is so reduced as to be only a thin diaphanous shell or membrane which permits ready diffusion of a milky nutritive fluid secreted by the uterine mucosa; in such forms the embryo appears to remain free in the uterine enlargement of the oviduct until birth. In other viviparous forms there is developed to a varying

degree an interrelated circulation between the embryo and the uterine mucosa of the mother. In some species the yolk-sac becomes applied to the uterine mucosa as the yolk is absorbed and there develop interdigitations between the two membranes, leading to the formation of a functional yolk-sac placenta; here nutrient material diffuses directly from the maternal blood in the uterine blood vessels to the foetal blood in the vessels of the yolk-sac. Each embryo develops its own placenta, but there appears to be great variation in the time and degree of placentation. The term "oviviparous" has been used to describe the mode of reproduction in *Chlamydose-lachus* and also in *Ginglyostoma*, in which it is entirely similar (Gudger, 1921); it has also been applied loosely in many other instances where the egg is retained in the oviduct or uterus for a variable time, but the available information does not permit accurate classification or description of these forms. (See Dean, 1906; Southwell and Prashad, 1919; Clark, 1922; Gudger, 1921; Smedley, 1926; Daniel, 1934; Gudger and Smith, 1933.)

The peculiarities of the mechanisms of reproduction in the *Elasmobranchii*, different as they are from all other fishes, suggest immediately that they are associated with the retention of urea in these forms. For if the urea is physiologically useful to the adult, it would seem possible that some supplementary or substitutional provision would have to be made for the developing embryo until such time as it is biochemically organised and can take care of itself.

Dakin (1911*a, b*) found that, whereas the plaice egg had only two-fifths of the osmotic pressure of sea water, the egg of *Scyllium canicula* has an osmotic pressure about the same as that of sea water. The immersion of the dogfish egg in a solution of lesser salinity resulted in a gain in weight due to absorption of water. He called attention to the fact that, relative to the respective osmotic pressures of the egg and the medium in which it is immersed, the eggs of the plaice and the dogfish resembled the adults. Schmidt-Nielsen and Schmidt-Nielsen (1923) have noted that the osmotic pressure of the ripe egg-yolks of *Spinax niger* and *Chimaera monstrosa* were the same as the blood sera, and the egg-case of the ray, according to Peyrega (1914), is permeable to both salts and water.

Krukenberg (1886, 1888) found urea to be present in considerable quantities in the egg-yolk and young embryos of *Mustelus laevis*, *Acanthias vulgaris*, *Scyllium canicula*, *Myliobatis aquila*, *Pristis antiquorum* and *Torpedo ocellata*. Of these only *Scyllium* is oviparous. Parker (1889) observed that urea is present in egg and pseudo-amniotic fluid of the viviparous *Mustelus antarcticus*, and Hunter and Dauphinée (1925) comment that they found this substance in the undeveloped eggs of *Squalus suckleyi*.

In a study on the utilisation of protein as a source of energy by the developing embryo, Needham and Needham (1930) have shown that a certain amount of urea is present in the undeveloped egg of the oviparous *Scyllium canicula* before the embryo has embarked on any appreciable metabolism of its own. The urea which the embryo forms during its development is only a contribution to a stock already provided by the maternal organs. The dogfish egg is closed at first, but about half-way through development slits appear at four corners of the egg-case which finally open

and allow sea water to penetrate. The egg-case itself is permeable to urea, but when eggs containing embryos from 1 to 1.39 cm. in length were placed in sea water there was a negligible escape of urea from the embryo to the exterior. The membranes that at this stage prevent diffusion are the bounding membranes of the yolk and embryo, so that no urea appears in the jelly or sea water between them and the shell. Needham (1926-7) introduced the word "cleidoic" to designate those eggs which are shut off from their surroundings as closed boxes. He points out that survival value attaches to the cleidoic egg, for the longer the embryo can continue its prenatal existence, the stronger it will be when it at length emerges, and for this end much more protection is required than for the minute egg whose development is more quickly accomplished. He calls attention to the fact that the only class of animals lower than the reptiles which have a cleidoic egg are the elasmobranchs.

Proceeding on the thesis that the end-products of protein metabolism are determined by the requirements of the embryo, Needham has argued that the elasmobranchs, having first discovered how to withstand severe uraemia (the reason for this uraemia not being stated), were able to enclose their embryos completely to permit the further development of the embryo; and then, having secondarily found it convenient to open the box again for respiratory or other purposes, but meanwhile having become adapted to this urea, they continued to store it within themselves. Needham (1929) applies this same argument to the birds, suggesting that the uric acid metabolism of these animals is also an adaptation to the cleidoic egg. Commenting on the superiority of uric acid over urea, Needham points out that urea could diffuse throughout the egg contents, reaching levels as high as 165 mg. per cent., which he considers to be a definitely "pathological" figure. If the avian embryo had to suffer from uraemia before hatching, he argues, natural selection would hardly have preserved it. The consequences of diffuse uraemia can be avoided by the use of uric acid, since it is much less diffusible and more easily confined to the allantoic fluid.

Needham's thesis that the form of nitrogenous excretion adopted by an animal depends principally on the conditions under which its embryo has to live seems to the author to be an inverted view of the matter. In point of fact, urea is one of the least toxic forms of nitrogen known, no ill effects being observed by its administration to mammals or fishes (Hugounenq and Florence, 1921) in amounts sufficient to raise the plasma level several hundred or even several thousand mg. per cent., providing water equilibrium is not simultaneously upset. (It is generally accepted that the pathological consequences of renal insufficiency in man are attributable to other factors—loss of protein in the urine, faulty excretion of salt and water, and perhaps the retention in the blood of some as yet unidentified toxic compounds—and that the retention of urea *per se* contributes little or nothing to the syndrome.) On the other hand, uric acid is very toxic for the higher animals and probably just as toxic for fishes.

We would consider the excretion of the uric acid in birds and reptiles as an adaptation of the adult to arid terrestrial life. Uric acid is physio-chemically a unique nitrogenous compound. It forms supersaturated colloidal solutions (Young

and Musgrave, 1932) from which it readily separates as an almost insoluble solid. It is apparently secreted into tubular urine of birds and reptiles in a concentrated solution from which, after the reabsorption of water and perhaps base by the cloaca, uric acid separates as a solid (Mayrs, 1924; Gibbs, 1929; Young and Dreyer, 1933; Marshall, 1934). Obviously the uric acid metabolism of birds and reptiles is a great advantage for arid, terrestrial life; on the other hand it would not seem, in view of the extremely low toxicity of urea, and the fact that other animals tolerate from 2000 to 4000 mg. per cent., that the mere diffusibility of this substance in the egg would determine the type of nitrogen metabolism in the adult.

With regard to the elasmobranch, it appears to be more in keeping with the facts to recognise that the cleidoic egg of these fishes is an adaptation secondary to the retention of urea. Having found that the retention of urea in the adult is valuable for osmotic reasons, these animals have found it convenient to enclose the embryo within a closed egg until such time as the membranes of the developing embryo can function to restrain the diffusion of the maternal gift of urea, as well as the complement added by its own metabolism.

This view explains why, as an alternative to the cleidoic egg, the Elasmobranchii have resorted to oviparous reproduction. Intra-uterine development will serve the purpose of protecting the young embryo against loss of urea better even than an egg-case. The superiority of viviparous over oviparous reproduction in respect to the retention of urea in the embryo may, in fact, account for the tendency among the more advanced forms to take up in increasing degree the former habit. Needham's explanation, on the other hand, throws no illumination on the fact that all elasmobranchs, excluding those having cleidoic eggs, are viviparous.

XII. THE DEVELOPMENT OF GLOMERULI.

It has been suggested that the renal glomeruli represent a primitive vertebrate character, and that the absence or degeneration of glomeruli in certain marine fishes is a specialisation associated with the decreased excretion of water, relative to the fresh-water ancestral stock (Smith, 1930*a*). This suggestion has been extended as a theory of evolution of the vertebrate kidney by Marshall and Smith (1930). With the secondary assumption of a marine habitat where the osmotic gradient is reversed and water excretion is greatly reduced, the teleost is at a disadvantage if glomerular activity is maintained at its full level, and there is consequently a need for reducing this activity to a minimum. Recent studies of glomerular activity in the marine sculpin by Clarke (1934) indicate that in the normal fish the rate of glomerular filtration is of the order of magnitude of 14 c.c. per kg. per day, in contrast to 200-400 c.c. per day in the fresh-water teleost. Under conditions of osmotic strain or in the state of physiological collapse studied by Grafflin (1931), the rate of filtration increases rather than decreases, rising from 14 to 100 c.c. per kg. per day, or more. It would appear that in the normal marine teleost glomerular activity is maintained by some physiological means at a reduced level, *i.e.* at a level considerably below the maximum. Whether this reduction is effected by chemical changes in the blood or by nervous control of the renal circulation is not known.

It is in line with the above interpretation that, whereas fresh-water fish show typically numerous and well-developed glomeruli, marine forms in general show a reduction in both number and size, and among the more highly specialised marine fishes many are known to have no glomeruli. Among the common aglomerular forms may be listed *Opsanus*, *Lophius*, *Syngnathus* and *Hippocampus*.

It is fairly certain that the recent elasmobranchs have been marine for a longer period of time than have any of the recent teleosts, and the question arises: if a marine habitat is favourable to the evolution of the aglomerular condition, why have not the elasmobranchs become aglomerular? Apart from the observations of Borcea (1906), Nash (1931), and Marshall and Smith (1930), there are no extensive observations on glomerular development in these fishes, but the data given by these investigators, combined with unpublished observations of the author on numerous eastern and western species, render it unlikely that an aglomerular elasmobranch will ever be found.

The answer to the above question would seem to lie in the fact that, because of its physiological uraemia, the elasmobranch has always enough water available to afford a moderate degree of glomerular activity and urine excretion. If it is faced with water shortage, the resulting anuria will automatically tend in time to correct the difficulty by promoting elevation of the osmotic pressure of the blood by urea retention. The osmotic strain of living in a marine habitat is thus shifted within the kidney, the process of urea conservation obviating the immediate need for water conservation, a minimum amount of water, at least, being always available for urine formation.

It is interesting in this connection to note that the elasmobranchs differ from the teleosts in showing a relative constant rate of urine formation (about 20 c.c. per kg. per day) as well as of glomerular filtration (about 80 c.c. per kg. per day), and that these figures are considerably above the corresponding rates observed in normal teleosts (3 and 14 c.c. respectively). Moreover, there appears to be no inhibition of renal activity in the normal animal; the observations of Clarke and Smith (1932, 1936) and Shannon (1934*a*, *b*) would indicate that a nearly maximal rate of renal activity is maintained at all times, or until such time as circulatory collapse leads to its reduction. This would suggest that, unlike the teleosts, the elasmobranchs may never have evolved mechanisms for reducing glomerular activity; because of their superior osmotic position, due in turn to their physiological uraemia, they have never been faced with the necessity for doing so.

XIII. SUMMARY.

The high urea content (2.0 and 2.5 per cent.) that characterises the blood, body fluids and tissues of the Elasmobranchii owes its origin to the relative impermeability of the gills and integument to this substance, and to the circumstance that the urea is actively conserved by the elasmobranch kidney.

In consequence of this physiological uraemia, the elasmobranch is osmotically superior to its environment, even in sea water, and is able to absorb at least a

minimum quantity of water for the formation of a urine that is isotonic or hypotonic to the blood, in accordance with the osmotic limitations of the fish kidney.

We may suppose that the uraemic state tends to develop and to be regulated more or less automatically; urea is constantly being formed by the ordinary metabolic combustion of protein; water shortage leads to oliguria and urea retention, and the accumulated urea in the blood raises the osmotic pressure of the latter to a point where water is again available by direct absorption. Water plethora (as in fresh water) leads to diuresis and increased urea excretion, which in turn lowers the osmotic pressure of the blood and in some measure, at least, reduces the rate of water absorption.

Trimethylamine oxide, which imparts about one-quarter as much osmotic pressure to the blood as does urea, is also conserved by the elasmobranch; the fact that this substance is present in the urine in lower concentration than in the blood suggests that, like urea, it is actively reabsorbed from the glomerular filtrate.

This physiological uraemia is apparently an archaic biochemical habit acquired early in elasmobranch evolution, since it is shared by the divergent orders of the subclass. Presumably it is a secondary mode of osmotic regulation superimposed upon the more primitive one of branchial regulation, as observed in the teleostomes.

The cleidoic egg, unique (among the fishes) in the Elasmobranchii, and the viviparous mode of reproduction, are viewed as adaptations to urea retention, protecting the embryo against the loss of urea during its early development.

Urea retention enables the Elasmobranchii to maintain a considerably greater rate of urine formation (water excretion) than is observed in the marine teleosts, a fact that perhaps explains why the former do not show the glomerular degeneration or the aglomerular development observed in the latter.

Whereas urine formation in the marine teleosts appears to be carried on normally at a reduced level considerably below the maximum possible rate, the elasmobranchs appear to maintain a maximal (though small) degree of glomerular activity at all times. Unlike the teleosts, they appear to possess no mechanisms for reducing glomerular activity; it may be that because of their superior osmotic position, due in turn to their physiological uraemia, they have never been faced with the necessity for conserving water to an excessive degree in the kidneys, and have therefore never evolved the means for doing so.

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THE GENETICAL CONCEPTION OF THE SPECIES

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I. INTRODUCTION.

THE title of this article naturally involves some consideration not only of what constitutes a species, but what, more particularly, constitutes a species in the genus *Gossypium*, since it is with the special application of the above title to this genus that I propose to deal.

There was a general discussion on the species concept at the Fifth International Botanical Congress (1930), out of which emerged a general consensus of opinion that it was not possible to define a species in all cases, and that if one did attempt to define one it had better be in as general terms as possible. In other words the

broaden the definition the better. Ostenfeld (1931) put forward the definition: "A species is a group of individuals which are alike in all the characters which we consider essential, provided that these characters are inheritable in the offspring derived after fertilisation." Vavilov (1931) stated: "A Linnean species is, according to our conception, a separate morpho-physiological system connected in its genesis with a definite environment and area." Hitchcock (1931) believed that the concept of most species must rest on the judgment and experience of the individual taxonomist who formed his opinion as to the limits of species largely from experience in the field, where he could observe large numbers of specimens and could note the variations in what he felt constituted a single species. Baker (1930) had earlier emphasised the point of view that the personal opinion of the taxonomist is of great importance. According to him, "no two authorities are likely to agree on the subject", and consequently he himself made it a rule to consider a group of individuals which are separated from all other groups by some definite combination of characteristics without intergrading as species; those that show intergradation as varieties. Calman (1930), alluding to the divergencies of opinion as to what constitutes a species, believes such divergencies somewhat over-estimated. He says: "I think it will be found that in most orders of animals there exists a considerable body of species regarding whose limits there is no serious difference of opinion among competent systematists, but alongside of these... a difficult residue in which delimitation of specific groups sometimes seems to be little more than a matter of personal taste."

In *Fanny's First Play* by George Bernard Shaw there is a discussion on whether the play was a good play. The dramatic critic's reply to this question was that if the play was by a good author it was a good play. The situation would appear to be somewhat similar in regard to what constitutes a species. There is, however, a consensus of opinion that there do exist natural groups of organisms differing from one another in numerous morphological and physiological peculiarities. As Jacot (1932) pointed out, no valid species has ever been based on a single character. "Any one species in a genus is differentiated from every other species by a set of morphological, as well also as physiological habitudinal characters." Extreme geographical variations, he says, are not considered as species, and are mostly referable to their specific type.

At the present time it is believed by all biologists that species, however we choose to define them, are descended from other species. This being so, it might be expected that some attempts would have been made to define the differences between species in terms of genes, instead of in terms of certain combinations of morphological characters. To assist in the delimitation of species, appeals have occasionally been made to other sciences, ecology, cytology, bio-geography, and even bio-chemistry, but genetical information has up to the present been very sporadic and often inconclusive.

A certain amount of information regarding the genetics of species crosses is, however, scattered through the literature, and it may be well to summarise this before passing on to a more detailed consideration of the situation in the genus *Gossypium*.

II. IDENTITY OF GENES IN DIFFERENT SPECIES.

(1) *The genus Drosophila.*

The genus *Drosophila* has been more extensively studied genetically than any other genus of living organisms. Morgan, Bridges and Sturtevant (1925) have discussed the question of corresponding and related genes in *Drosophila* species. They point out that related species must have many genes in common, and are in fact similar only because of this group of common genes. Unfortunately there is only one interspecific cross possible in *Drosophila*, viz. *D. melanogaster* \times *D. simulans*, and here, although the two species are closely similar morphologically, the hybrids are completely sterile. By crossing the recessive mutants found in both the species with the corresponding wild type, it was established that sixty-five recessive mutant genes of *melanogaster* in fifty-four distinct loci were recessive also in interspecific hybrids. Twenty-three recessive mutants in *simulans* gave similar results. Although the crosses cannot be pursued beyond the F_1 it was definitely shown that each species carries the dominant allelomorphs for the mutant genes of the other species as well as for its own mutants. A comparison of the chromosome maps reveals great similarity in the two *X*-chromosomes, both in the order of the parallel mutants, and even in the percentage of crossing-over. In chromosome III, however, the sequence of mutant genes and the cross-over values are strikingly different.

Although other species of *Drosophila* will not cross with *melanogaster*, many of the sex-linked mutants of *obscura*, *Willistoni* and *virilis* closely resemble sex-linked mutants of *melanogaster*, and though no direct proof of identity of loci is possible, it is a not improbable assumption that many of these resemblances are due to some genic homology. Sturtevant (1921), discussing *Drosophila* species, says: "Species, then, differ from each other in many genes. The differences though numerous are such that each produces only a slight effect on the organism. These differences are of the same kind as are the mutational differences and may be supposed to have arisen by mutation."

(2) *The genus Cavia.*

Detlefsen (1914) crossed the wild Brazilian guinea-pig (*Cavia rufescens* Lund.) with the common domestic guinea-pig (*C. porcellus* Linn.). The F_1 was sterile on the male side, and the males of the first back-cross to the domesticated species were also sterile. In subsequent further back-crosses fertility of the males was ultimately completely established.

Detlefsen established that the light agouti of the domestic type was completely dominant over the dark agouti of the wild species, and allelomorphic to it. That is, the wild species possessed a different allele in respect of the agouti character.

(3) *The genera Zea and Euchlaena.*

Zea (the common maize) is a monotypic genus, and the large Mexican grass teosinte (*Euchlaena mexicana*) is its nearest wild relative. Although placed in different genera, maize and teosinte cross readily (Collins, 1919) and give rise to a complete series of intermediates "graduated to any desired degree of minuteness".

Collins and Kempton (1920) investigated a cross between a small variety of popcorn maize and teosinte. "The F_1 plants showed characters which, for the most part, were intermediate between those of the parents. The F_2 plants were also intermediate, with a greatly extended range of variation. Thirty-three of the characters that differentiate the parents were chosen and recorded for each of the one hundred and twenty-seven F_2 plants. The distribution of these characters, with one or two exceptions, showed little or no evidence of alternative or Mendelian inheritance. With respect to individual characters, the extreme variants approached, and in some instances exceeded those of the parents, but none of the plants possessed any large numbers of the characters of either maize or teosinte."

Kempton (1924) studied the mode of inheritance of certain maize recessives in the same intergeneric cross. He was able to show that the three recessives crinkly, ramose, and brachytic behaved also as recessives in crosses with teosinte, and reappeared in F_2 . The ratio of dominant to recessive was 3 : 1 with crinkly and ramose, but about 8 : 1 with brachytic. Classification of ramose was not difficult, but brachytic segregates in F_2 resembled normals much more than in intermaize hybrids, and the author states: "There seems little doubt that modifying factors affecting stature have been introduced through the teosinte parent."

In the cross of the maize recessive "golden" by teosinte, yellow plants appeared in F_2 , although there was no clear distinction between golden and green. Here again the author suggests that *Euchlaena* possesses modifying factors, affecting the expression of the golden green factor pair.

It is important to note here that genetic investigation did not cease with the recording of the mere fact of blending inheritance. By making use of strongly marked maize recessives it was clearly established that the dominant allelomorphs of these recessives were present also in teosinte, and that where continuous gradation occurred, as between golden and green, it could be attributed to the effect of modifiers introduced from teosinte.

In considering the results of the maize-teosinte crosses described above, too much importance should not be ascribed to the fact that these two plants have been placed in different genera. It was shown by Kuwada (1919) that both *Euchlaena* and *Zea* and the F_1 between them have the number 10 as the haploid chromosome number, and it was later found by Longley (1924) that an F_2 plant of the same cross showed no irregularity in chromosome distribution in pollen mother cell development. It is thus merely a matter of taxonomic convenience that these two species are placed in different genera, since in spite of the great morphological differences existing between them they are genetically closely alike.

Emerson (1929) described the results of crosses between maize and a different species of teosinte (*Euchlaena perennis*). This species has 20 pairs of chromosomes instead of the 10 pairs of *mexicana*. In this cross the maize parent carried five dominants, and the F_1 was back-crossed to a maize type which carried the recessives of these five. Emerson, discussing the results, states: "Great difficulty was experienced in classifying seeds involving the C c pair. Many of the seeds classed as coloured were white except for a few spots of colour and some classed as white,

c, may well have been genotypically coloured, **C**; one plant, of five tested from seeds classed as white, proved to be **C c**. Some difficulty was experienced also in classifying seeds involving **Y y** and **B B^w**, and appropriate tests showed that at least one seed classed as white **y** was genotypically yellow **Y y**."

This experiment is not so valuable as the preceding, since both the dominants and recessives came from maize, and their inheritance is merely being followed in a genotypical milieu in which a set of teosinte genes are also concerned.

The inheritance of four dominant teosinte genes was followed in crosses with maize recessives. It was found that in respect of the four genes **G**, **Lg**, **Su**, and **Wx** the back-cross could be correctly classified except for the pair **Su su**. There was, however, great deficiency in the number of plants carrying the recessive maize genes.

(4) *The genus Antirrhinum.*

Baur (1932 and earlier papers) observed that in the F_2 of a cross between two wild species occurred an absolutely boundless series of colours and forms, and among thousands of individuals hardly two could be found alike. In crosses of cultivated races of *Antirrhinum majus* with wild species, F_1 showed dominance of some characters and intermediacy of others. In F_2 an enormously motley segregation took place, as in the crosses between the wild species. Baur concluded that more than 100 genes must be segregating.

For the purpose of this review, what is important is that all the wild species appear to contain the dominant allelomorphs of most of the cultivated recessives. If, for example, a cross was made between *A. latifolium* (wild) and *A. majus* (cultivated) in which the *latifolium* parent bore the dominant fuchsin flower colour, and *majus* eosin red flower colour, the F_1 was fuchsin and in F_2 a quarter of the plants were eosin red.

Baur (1932) provides further information respecting the relationship of genes in the genus *Antirrhinum*. The cross of *A. majus* (viridiflora, elfenbein, delila) by *A. hispanicum*, back-crossed with the triple recessive *A. majus*, gave a majority of plants in which the triple dominants were practically indistinguishable from triple dominants in *majus*. Extracted triple dominants were similarly indistinguishable from those of homozygous *majus*. This indicates that the genes **Vir-Inc-Del** of *A. hispanicum* must be identical in their effects with the same genes in *majus*.

Certain species of *Antirrhinum* possess the Alpine habit, i.e. growth is adpressed. This is the case in *A. glutinosum* and *A. molle*. The F_2 of a cross between these species consists only of adpressed plants, indicating that the genetic basis for this character is very similar in these two species. Similarly the small leaves of *A. Barrelieri* and *A. tortuosum* are essentially genetically identical. That is, morphologically similar characters in various wild species are often genetically conditioned in a similar manner. The same holds good also for self-sterility factors.

The main colour genes of *majus*, such as **Del**, **Eos**, **Dil**, **Pal**, **Niv**, play no part in many of the wild species. The gene **sulf**, for example, characterises all the races of *latifolium*.

Interspecific hybrids give rise to an extremely varied array of types. Many of these are unbalanced and cannot survive in nature, *e.g.* they are very slow growing or susceptible to disease, or they easily succumb to unfavourable conditions. A small percentage only are really effective combinations.

(5) *The genus Tephrosia.*

Harrison (1920) observed that in crosses within the limits of the lepidopterous species *Tephrosia crepuscularia*, inheritance of melanism exhibited simple monohybrid segregation. In the interspecific cross *T. crepuscularia* \times *T. bistortata* a nondescript series of intergrades results in F_2 in respect of the same character. Many examples of this phenomenon will be cited for the genus *Gossypium*, and also what is believed to be the correct explanation.

(6) *The genus Collinsia.*

Hiorth (1933) in a study of hybrids between *Collinsia bicolor* ($n=7$) and *C. bartsiaefolia* ($n=7$) showed that the cross is difficult to make and is almost sterile. By repeated back-crossing it was found possible not only to transfer *bartsiaefolia* genes to *bicolor* and *vice versa*, but also to introduce a section of a *bartsiaefolia* chromosome into *bicolor* by four back-crosses to the latter. Although segregation ratios were irregular, crossing-over involving the *bartsiaefolia* segment was shown to occur. *The gene W (violet flower colour) of bartsiaefolia was established to be different from the corresponding W gene of bicolor, behaving as a weaker allele.*

(7) *The genera Platypoecilus and Xiphophorus.*

Kosswig (1929) investigated the intergeneric cross *Platypoecilus* \times *Xiphophorus*, two species of cyprinodont fish. He showed that certain colour genes in the Z-chromosome of *Platypoecilus* had their effects greatly augmented in the cross with *Xiphophorus*. This was particularly the case with the *nigra* gene, which in *Platypoecilus* produces a wedge of colour from the tail running towards the head. In F_1 of the intergeneric cross the colour was stronger and more widely distributed, sometimes nearly covering the whole body. In the back-cross of F_1 with *Xiphophorus* a further increase in amount of pigmentation occurred and some individuals possessed the melanic pigmentation so strongly that pathological melanotic tumours developed.

Two other genes were found to segregate normally in the intergeneric cross, *viz.* S, black spot on tail fin of *Platypoecilus*, and f, a recessive autosomal gene of *Platypoecilus* which inhibits the production of melanin.

(8) *The genus Nicotiana.*

Clausen (1928) suggested that the origin of *Nicotiana tabacum* may be ascribed to doubling of the chromosome number in a hybrid between *N. sylvestris* and *N. tomentosa* or close allies of these species, followed by secondary alterations in

factorial constitution and organisation. The same author (1932) made an attempt to determine experimentally the extent to which homologous chromosomes of *sylvestris* and *tabacum* were comparable in genetic content.

He transferred the *P*-chromosome of *sylvestris* to *tabacum* by the method of repeated back-crossing, and was able to show that in respect of two factors, **Cn** and **pk**, the *P*-chromosome of *sylvestris* was genetically homologous with the *P*-chromosome of *tabacum*.

III. GENERAL CONCLUSIONS RESPECTING THE BEHAVIOUR OF GENES IN INTERSPECIFIC CROSSES.

We have now discussed the behaviour of interspecific and of some reputedly intergeneric crosses with particular reference to the genes themselves. The most striking point is that if we are able to cross two species at all, or two species even from different genera, and obtain sufficient fertility for the conductance of genetic experiments we always get evidence that either identical or at least homologous genes lie in homologous loci in the species involved. We may set forth the generalisations thus:

(1) Allelomorphic relationships exist between the genes of two or more crossable species in the same genus, or sometimes in species regarded by taxonomists as generically distinct. This usually means that mutants in one species are represented by dominant allelomorphs in other species of the genus (*e.g.* *Zea-Euchlaena*, *Antirrhinum*, *Platypocilus-Xiphophorus*, *Nicotiana*, *Cavia*, *Collinsia*, *Gossypium*).

(2) Even in crosses which are not fertile beyond the F_1 (*e.g.* *Drosophila melanogaster* \times *D. simulans*) alleles of the mutants of both species exist in these two species, and the similarity of the *X*-chromosome map in these two and other species of the genus *Drosophila* leads to the same conclusion, viz. that a large number of loci are common to many of the species in the genus.

(3) Genes can be transferred from one species to another by repeated back-crosses, even if sterility of F_1 is initially very great. In this case the species concerned must again be assumed to hold loci in common. When so transferred the dominant genes may be identical (*Antirrhinum*, *Nicotiana*), or allelomorphic, *i.e.* similar but not identical (*Collinsia*, *Cavia*).

(4) In one case only did the introduction of one gene into the genotype of another species (*Platypocilus-Xiphophorus*) lead to marked changes of a pathological kind in developmental physiology.

(5) Genes which exhibit simple monohybrid relationships within the species show complicated and often unanalysable blending inheritance in interspecific crosses (*Tephrosia*).

Consideration of the existing data, admittedly scanty, leads to the conclusion that in the evolution of one species from another, gene substitution is the one process which we can see and analyse. When, for example, it can be shown that in two species morphologically and physiologically so diverse as *Zea mays* and *Euchlaena mexicana*, twenty-eight maize genes were represented by dominant or recessive allelomorphs

in *Euchlaena*, we suspect that taxonomic divergence is correlated with an increasing tendency to change the genes from an identical to an allelomorphous state. This question will be further considered in the discussion on *Gossypium*.

IV. MODIFYING GENES.

The early history of genetic investigation for the first decade of the twentieth century concerned itself chiefly with pairs of allelomorphs the phenotypic effects of which manifested themselves in marked discontinuities. While the foundations of genetical theory were being laid, and while the intricacies of various types of factorial interaction were still to be worked out, it was not possible fully to realise the enormous variation in phenotypic effect which could result from segregation involving merely single allelomorphous pairs. Nevertheless, the fact was noted by Cuénot (1904) cited by Bateson, Saunders and Punnett (1905) that, by crossing albino mice of different extraction, the allelomorphs determining the various colours will themselves segregate in the gametogenesis of the albino in ordinary Mendelian fashion. This demonstrated segregation of one or more pairs of allelomorphs producing zero phenotypic effect in a given genotype.

That the inheritance of a complex character could be determined by factors responsible for minute heritable discontinuities was envisaged by Bateson and his co-workers in the classical *Reports to the Evolution Committee of the Royal Society*. The point of view held by Bateson is shown clearly in the following quotations (1902, 1905): "At first sight it seems that cases of continuous variations, blending in their hereditary transmission, form a class apart from those to which Mendel's principles apply.... It must be recognised that in, for example, the stature of a civilised race of man, a typically continuous character, there must certainly be on any hypothesis more than one pair of possible allelomorphs. There may be many such pairs.... If there were even so few as, say, four or five pairs of possible allelomorphs, the various homo- and heterozygous combinations might, on seriation, give so near an approach to a continuous curve, that the purity of the elements would be unsuspected, and their detection practically impossible."

Referring to investigations on the silkworm, Bateson, Saunders and Punnett (1905) say: "The character called by Coutagne, *richesse de soie*, shows to all appearances continuous variation and a non-Mendelian inheritance, not undergoing any sharp gametic segregation, and being capable of intensification by gradual selection. It is likely that this quality depends on numerous factors."

Practically no progress in the study of factor differences with minute effects was made until the Morgan school of *Drosophila* workers not only made it possible to demonstrate the existence of what came to be termed "modifying factors", but actually also to indicate the location of some of them on the chromosome map.

Dexter (1914), in an analysis of the beaded wing character in *Drosophila*, established the existence of a factor which when present increased the amount of beading. Bridges (1916, 1919) found no less than eight factors modifying the mutant eye colour eosin. Seven of these diluted the colour and one darkened it. It was

possible to isolate stocks which varied from a shade darker than eosin down to a white indistinguishable from the white determined by the factor *W*, an allelomorph of eosin. In each of the eight grades of colour differing from eosin the factor for eosin, *W*^e, was accompanied by a specific modifier which produced a definite augmentation or reduction in the degree of coloration. Some of these modifiers, the factors pinkish, cream *b*, and cream III, were accurately located by the customary methods employed in *Drosophila* studies. The case of the specific eosin modifiers was recognised as of extreme importance by Jennings, who pointed out that studies of *Drosophila* were furnishing all that could be asked as to the existence of a single unit character in a series of numerous hereditary gradations. He says (1917): "To sum up, it appears to me that the work in Mendelism, and particularly the work on *Drosophila*, is supplying a complete foundation for evolution through the accumulation by selection of minute gradations."

A detailed study of the genetic effects of long-continued selection was carried out by Sturtevant (1918) on bristle number involved in the character "dichaete" of *Drosophila*. By selection, plus and minus lines were obtained; linkage tests were made, and it was demonstrated that at least two pairs of modifying factors for bristle number were present in selected lines in both the second and third chromosomes.

It is unnecessary to cite in detail the large number of subsequent studies demonstrating the existence of modifiers producing minute but measurable effects, since the main reason for discussing the whole question of modifiers will now be clear. The type of segregation encountered in crosses of species or even geographical races is usually of the blending type, explicable on the hypothesis that modifying factors are concerned. Since complicated cases of modifier interaction have been worked out in *Drosophila*, the genetics of which is understood in broad outline, it is highly probable that the intricate systems of modifiers embodied in species will in time be reduced to order.

V. COMPARATIVE GENETICS OF THE GENUS *GOSSYPIMUM*.

In order to investigate the comparative genetics of a group of species in a genus, certain prerequisites are necessary. First the species must be good species—recognised as such by competent taxonomists; second, they should differ in a large number of their characters; third, although some sterility may be permissible it should not be so complete as to preclude genetical analysis; fourth, conjugation of chromosomes should take place normally, *i.e.* cytological abnormalities should not intervene.

The genus *Gossypium*, the taxonomy and genetics of which have been summarised by myself (Harland, 1932*d*) and to which the important cultivated cottons belong, provides material admirably fulfilling the above criteria of suitability for interspecific genetical analysis. This genus is divided into a 13-chromosome and a 26-chromosome group of species, with great diversity of characters. The 13-chromosome group contains at least eleven wild species from Asia, Africa, Australia, North America and the Galapagos Islands. The 26-chromosome group, with which

my investigations have been specifically concerned, comprises the following wild and cultivated species:

- Central America: *G. hirsutum* L. (cultivated annual Upland cotton).
G. purpurascens Poir. (cultivated and wild of Haiti and lesser Antilles).
G. barbadense L. (cultivated and wild kidney but includes also the annual Egyptian and Sea Island).
- South America: *G. purpurascens* Poir. (cultivated cottons of North Brazil).
G. barbadense L. (cultivated cottons of Peru).
- Galapagos Islands: *G. Darwinii* Watt (wild) endemic.
- Polynesia: (a) *G. tomentosum* Nutt. (endemic to Hawaiian Islands).
 (b) *G. taitense* Parl. (endemic to Fiji and the Marquesas).

It has recently been established by Skovsted (1934) working in my laboratory that the New World species of *Gossypium* with 26-chromosome pairs, are probably allopolyploids. They possess two subgenoms of 13 chromosomes each, one cytologically identical or nearly so with cultivated *G. arboreum* and *G. herbaceum* of Asia, and the other belonging to an as yet undetermined 13-chromosome species.

It is a fact of extreme interest that three out of six species are endemic in widely separated Pacific Island groups, and from this it has been argued (Harland, 1935 a) that the New World allopolyploids probably arose in late Cretaceous times in the eastern part of a land connection between South America through Polynesia to the East Indies. Whether this supposition be true or not, it is certain that the endemic species *Darwinii*, *taitense* and *tomentosum* have been separated from their South American congeners for an interval of time only to be estimated geologically—certainly for several million years, and that the genetical consequences of such isolation can be followed experimentally in crosses between these species and the present-day South American species. A detailed study of the two American species *hirsutum* and *barbadense* has revealed morphological and physiological differences no less profound, and although much information has been accumulated regarding the genetical architecture of all six New World species, most attention has been paid to crosses involving *G. hirsutum* L. and *G. barbadense* L. represented among cultivated cottons by Upland and Egyptian cottons respectively.

The main question to which an answer has been sought may be stated thus: What are the genetic bases of the differences between the two good species *hirsutum* and *barbadense*, which are profoundly separated in respect of practically every morphological and physiological character, and which have been isolated in different geographical areas over a long period of time?

VI. METHODS OF INVESTIGATION.

Our experiments began in 1926 at the Trinidad Cotton Research Station. It was found that most of the scanty work previously done on the genetics of either Upland or Egyptian Sea Island cotton had been carried out in interspecific crosses.

The usual complex type of blending inheritance had been encountered, and even simple pairs of characters such as yellow-cream corolla or petal spot which in intervarietal crosses had been shown to behave in simple monohybrid fashion (Harland, 1916, 1918, 1920; Kearney, 1924) proved to be very complicated when followed out in *hirsutum*-*barbadense* crosses.

Further work (Harland, 1929-34) indicated that *all characters* exhibiting simple monohybrid behaviour *within* the species exhibited complicated and often continuous blending in the F_2 of the interspecific cross *barbadense* \times *hirsutum*. In some characters such as "crinkled dwarf", a characteristic mutant which has occurred several times in *barbadense* but not in *hirsutum*, blending in the interspecific F_2 was extreme and the expression of the mutant character varied from an extreme and exaggerated form to one in which the expression of the crinkled character was only slight.

In other characters, such as the red plant body R^B of *barbadense*, segregation into red and green could be fairly easily followed in F_2 , though some of the reds exhibited coloration to a very slight degree.

As a working hypothesis it was assumed that the clear segregation of a factor pair in an intraspecies cross resulted from the fact that the rest of the genotype provided a relatively constant genetical background upon which both the dominant and recessive phases of a gene were clearly distinguishable. If, however, the whole gene complex (genotype) of another species were markedly different, we should expect in the second generation of an interspecific cross a large number of different genetical backgrounds upon which the given pair of alleles would manifest themselves. Unless dominant and recessive could be clearly separated on all possible combinations of genes in the gametogenesis of an interspecific cross, blending inheritance would result, and sharp delimitation into the expected two or three classes of typical monohybrid segregation would not exist.

The assemblage of genes which singly or in combination with other genes are capable of affecting the degree of expression of a given pair of alleles may be termed a "modifier complex", and if the hypothesis is tenable—that species differ to a greater or less extent in modifier complexes—it should be possible by providing a uniform genotypic background to convert a case of blending inheritance into a simple case of clear-cut monohybrid inheritance. It has been demonstrated that this can be done by a sufficient number of back-crosses of the heterozygote to either or both parental species. Usually it has proved better to cross the heterozygote to the species introducing the dominant, since the latter almost always carries a modifier complex which has the effect of enhancing the effect of the dominant allele.

The discovery that it was possible in species crosses to eliminate the confusing effects of differing modifier complexes on the segregation of pairs of alleles led to a series of experiments in which genes were transferred by repeated back-crossing from one species to another. In the course of these experiments some knowledge of the genetical architecture of the different species of the New World tetraploid *Gossypiums* has been arrived at.

This knowledge, which principally concerns the two species *barbadense* and *hirsutum*, may be presented in the form of a number of generalisations, each of

which will receive adequate discussion. Information regarding the genetical make-up of the other four species of the allopolyploid group will also be given.

VII. GENERALISATIONS REGARDING THE GENETICAL CONSTITUTION OF *G. BARBADENSE* L. AND *G. HIRSUTUM* L.

The same gene or an allele of it is common to both species.

Table I. *Distribution of genes in Gossypium hirsutum and G. barbadense.*

Gene	Effect	<i>hirsutum</i>	<i>barbadense</i>
RH	Red plant body	Found but rare	n.r.
rH	Green plant body	p.f.	p.f.
RB	Red plant body	n.r.	Found but rare
rB	Green plant body	p.f.	p.f.
SH	Petal spot	Found but rare	n.r.
SB	Petal spot	n.r.	p.f.
s	No petal spot	p.f.	Found but not common
YB	Yellow corolla	Yellow corolla recorded but rare. Probably stronger allele of YB	p.f.
yB	Cream corolla	p.f.	Found but not common
yD	Cream corolla	p.f.	p.f.
P	Yellow pollen	Found but not common	p.f.
p	Cream pollen	p.f.	Found but not common
N	Naked seed	Found but not common	n.r.
n	Fuzzy seed	p.f.	Character recorded but relationship to <i>hirsutum</i> fuzzy unknown
T	Tufted seed	Found rarely but relationship to <i>barbadense</i> tufted unknown	p.f.
t	Naked seed	n.r.	Found but not common
F	Fuzzy seed	p.f.	Character recorded but relationship to <i>hirsutum</i> fuzzy unknown
f	Tufted seed	Found rarely but relationship to <i>barbadense</i> tufted unknown	p.f.
Cha	Green	Found but not common	p.f.
cha	Chlorophyll deficient	p.f.	n.r.
Chb	Green	p.f.	n.r.
chb	Chlorophyll deficient	n.r.	p.f.
ON	<i>hirsutum</i> leafshape	p.f.	Probably not present
Oo	Okra leafshape	Not uncommon mutant of ON	n.r.
OS	Superokra leafshape	Rare mutant of Oo	n.r.
OB	Sea Island leafshape	Probably not present	p.f.
Co	Contorta leaf	n.r.	Rare dominant mutant
co	Normal leaf	p.f.	p.f.
CRB	Normal leaf	n.r.	p.f.
CRH	Normal leaf	p.f.	n.r.
CRM	Normal leaf	Found but prevalence not known	n.r.
cR	Crinkled leaf	n.r.	Rare mutant of CRB
KB	Khaki lint	n.r.	Fairly common
KH	Khaki lint	Found but rare	n.r.
k	White or cream lint	p.f.	p.f.
GL	Green lint	Found but very rare	n.r.
g ^L	White or cream lint	p.f.	p.f.
V	Green leaf	p.f.	Prevailing form but may be different allele of V
v	Virescent yellow leaf	Rare mutant	n.r.

Note. n.r. (not recorded) means that the gene is not present in a considerable number of varieties examined. p.f., prevailing form.

In Table I will be found summarised the mode of distribution of all genes so far investigated in *barbadense-hirsutum* crosses. The genes may be divided into distinct classes as follows:

- A. Dominants found only in *hirsutum*:
 - (1) Rare R^H, S^H, N, K^H, G^L .
 - (2) Common F, C^{HB}, C^{RH} .
- B. Dominants found only in *barbadense*:
 - (1) Rare R^B, K^B .
 - (2) Common S^B, T, C^{RB} .
- C. Recessives found only in *hirsutum*:
 - (1) Rare c^{ha}, v .
 - (2) Common —.
- D. Recessives found only in *barbadense*:
 - (1) Rare t, c^r .
 - (2) Common c^{hb} .
- E. Dominants found in both *hirsutum* and *barbadense*:
 - (1) Prevailing form in *barbadense* but uncommon in *hirsutum* Y^B, P, C^{ha} .
 - (2) Prevailing form in both species, V .
- F. Recessives found in both *hirsutum* and *barbadense*:
 - (1) Prevailing form in both $r^B, r^H, c^o, k^B, k^H, g^L, y^D$.
 - (2) Prevailing form in *hirsutum* but uncommon in *barbadense* s, y^B, p .
- G. Genes exhibiting intermediacy in heterozygous state found only in *hirsutum*:
 - (1) Rare O^O, O^S .
 - (2) Prevailing O^N .
- H. Genes exhibiting intermediacy in heterozygous state found only in *barbadense*:
 - (1) Rare C^o .
 - (2) Prevailing O^B .

The most important points emerging from a survey of Table I are as follows: There is no definite wild type in either species. The commonest form of genotype of the two species is as follows:

hirsutum: $r^B r^B r^H r^H ss y^B y^B y^D y^D pp FF c^{ha} c^{ha} C^{hb} C^{hb} O^N O^N c^o c^o C^{RH} C^{RH} k^B k^B k^H k^H g^L g^L VV$.

barbadense: $r^B r^B r^H r^H S^B S^B Y^B Y^B y^D y^D PP TT C^{ha} C^{ha} c^{hb} c^{hb} O^B O^B c^o c^o C^{RB} C^{RB} k^B k^B k^H k^H g^L g^L VV$.

Here it will be seen that the two species are apparently alike in constitution in $r^B r^B, r^H r^H, y^D y^D, c^o c^o, k^B k^B, k^H k^H, g^L g^L$ and VV , i.e. in seven recessives and only one dominant. In respect of the other genes the commonest situation is that at a given locus there is a series of multiple alleles, some of which characterise *hirsutum* and some *barbadense*. In no case where a multiple allele series has been

established is a dominant common to both species. In the case of the single dominant gene **V**, which is apparently the predominant allele in both species, there is some doubt whether the *barbadense* gene is not a weaker allele at the same locus.

The apparent identity of seven recessive genes in the two species may also be illusory, since a given recessive might be phenotypically similar in regard to its main manifestation but differ in some other respect. Timoféeff-Ressovsky (1932b) has shown in X-ray studies of the mutability of the white allele of *Drosophila menologaster* that there exist two different morphologically indistinguishable normal alleles in the white series which can only be distinguished by their rate of mutation. In further researches on the vitality and fertility of various white alleles, the same author (1933) has shown that the two "normal" white alleles can be distinguished by their relative vitality. That is, there exist "kleine Vitalitätsmutationen" without visible morphological effect, within the white allele series.

Considerations such as these render it necessary to conduct detailed studies on the additional physiological manifestations of the alleged common recessives.

It is highly probable, however, that *hirsutum* and *barbadense* possess relatively few genes in common, and that geographical isolation over a long period of time has resulted in the production of new alleles at most loci and in a characteristic distribution of the differing alleles between the two species.

VIII. GENETICAL CONSTITUTION OF *PURPURASCENS*, *TAITENSE*, *TOMENTOSUM* AND *DARWINII*.

In discussing the genetical constitution of the four remaining species of *Gossypium* it is necessary to bear in mind the fact that relatively few types of *taitense*, *tomentosum* and *Darwinii* were available. In the case of both *taitense* and *tomentosum* our original stock of seed was from a single plant, while only four single plant samples of *Darwinii* were available. A wider range of *purpurascens* types was worked with, but examination of the species throughout the whole geographical range would no doubt reveal greater variability.

The distribution of the known genes is set out in Table II.

The commonest genetical constitution of the four species may be set out as follows:

purpurascens: $r^{HrH} r^{BrB} r^{DrD} Y^{BYB*} y^{DyD} SS* PP OO* C^{ha}C^{ha} C^{hb}C^{hb} c^oc^o C^{RcR*} k^{HkH} k^{BkB} g^{LgL} VV h^{ThT} h^{BhB}$.

taitense: $r^{HrH} r^{BrB} r^{DrD} y^{ByB} y^{DyD} SS* pp OO* c^{ha}c^{ha} C^{hb}C^{hb} c^oc^o C^{RcR*} K^{HkH} k^{BkB} g^{LgL} VV h^{ThT} h^{BhB}$.

tomentosum: $r^{HrH} r^{BrB} r^{DrD} Y^{BYB*} y^{DyD} ss PP OO* C^{ha}C^{ha} C^{hb}C^{hb} c^oc^o C^{RcR*} k^{HkH} k^{BkB} K^{Tkt} g^{LgL} VV H^{ThT} h^{BhB}$.

Darwinii: $r^{HrH} r^{BrB} R^{DRD} y^{ByB} y^{DyD} SS* PP O^{DO}D* C^{ha}C^{ha} c^{hb}c^{hb} c^oc^o C^{RcR*} k^{HkH} g^{LgL} VV$.

* Allele of locus in *hirsutum* or *barbadense* demonstrated but exact identity uncertain.

Table II. The distribution of genes in four species of *Gossypium*.

Gene	Effect	<i>purpurascens</i>	<i>taitense</i>	<i>tomentosum</i>	<i>Darwini</i>
R ^H	Red plant body	n.r.	n.r.	n.r.	n.r.
r ^H	Green plant body	p.f.	p.f.	p.f.	p.f.
R ^B	Red plant body	Common in West Indies form	n.r.	n.r.	n.r.
r ^B	Green plant body	p.f.	p.f.	p.f.	p.f.
R ^D	Red plant body	n.r.	n.r.	n.r.	Found in one type, prevalence not known
r ^D	Green plant body	p.f.	p.f.	p.f.	p.f.
Y ^B	Yellow corolla	Character present but probably due to another allele	n.r.	Character present but probably due to another allele	n.r.
y ^B	Cream corolla	Rare	p.f.	n.r.	n.r.
Y ^D	Yellow corolla	n.r.	n.r.	n.r.	p.f.
y ^D	Cream corolla	Rare	p.f.	n.r.	n.r.
S ^B	Petal spot	Allele present but not known whether identical with S ^B or S ^H	Apparently represented by another weaker allele	n.r.	Allele present, probably different from S ^B or S ^H
S ^H	Petal spot	Rare	n.r.	p.f.	n.r.
s	No petal spot	Rare	n.r.	p.f.	n.r.
P	Yellow pollen	p.f.	n.r.	p.f.	p.f.
p	Cream pollen	Rare	p.f.	n.r.	n.r.
ON	<i>hirsutum</i> leafshape	Allele present, not certain if identical	Allele present, not certain if identical	Allele present, not certain if identical	Allele present, not certain if identical
O ^O	Upland okra	Occurs rarely	n.r.	n.r.	n.r.?
O ^S	Upland super-okra	n.r.	n.r.	n.r.	n.r.
O ^P	Laciniated leaf <i>purpurascens</i>	Rare	n.r.	n.r.	n.r.
O ^D	Laciniated leaf <i>Darwini</i>	n.r.	n.r.	n.r.	Occurs
O ^B	Sea Island leafshape	n.r.	n.r.	n.r.	n.r.
Ch ^a	Green	p.f.	n.r.	p.f.	p.f.
ch ^a	Chlorophyll deficient	Rare	p.f.	n.r.	n.r.
Ch ^b	Green	p.f.	p.f.	p.f.	n.r.
ch ^b	Chlorophyll deficient	n.r.	n.r.	n.r.	p.f.
C ^o	Contorta leaf	n.r.	n.r.	n.r.	n.r.
c ^o	Normal leaf	p.f.	p.f.	p.f.	p.f.
CR	Normal	Allele present	Allele present	Allele present	Allele present
c ^R	Crinkled leaf	n.r.	n.r.	n.r.	n.r.
K ^B	Khaki lint	?	n.r.	n.r.	?
K ^H	Khaki lint	?	Found	n.r.	?
K ^T	Khaki lint	n.r.	n.r.	p.f.	?
k	White or cream lint	p.f.	n.r.	n.r.	n.r.
GL	Green lint	—	—	—	—
g ^L	White or cream lint	p.f.	p.f.	p.f.	p.f.
V	Green leaf	p.f.	p.f.	p.f.	p.f.
v	Virescent yellow leaf	n.r.	n.r.	n.r.	n.r.
HT	Hairy plant body	n.r.	n.r.	p.f.	?
h ^T	Glabrous plant body	p.f.	p.f.	n.r.	?
H ^B	Hairy plant body	n.r.	n.r.	n.r.	?
h ^B	Glabrous plant body	p.f.	p.f.	p.f.	?

Note. n.r. (not recorded) indicates that the gene was not present in all types of the species which it was possible to examine. p.f., prevailing form.

The main points regarding the above genetical formulae may be briefly discussed.

(1) There are no well-marked differences between *purpurascens* and *hirsutum* in genetical composition with respect to the major genes worked with. Nevertheless it is fairly clear that the genes of *purpurascens* are not always identical with those of *hirsutum*, although these two species may be somewhat allied. Morphologically the differences between *purpurascens* and *hirsutum* are wide, and there is some sterility in the F_2 of the interspecific cross.

In the case of **Y**, **S**, **O**, and **G^R** it is probable that *purpurascens* has special alleles differing from those of *hirsutum* or *barbadense*.

(2) *Taitense* is closely allied morphologically to both *purpurascens* and *hirsutum*. Some of the genes are probably peculiar to this species, but insufficient work has been done to state this with certainty.

(3) *Tomentosum*, as one might imagine from its occurrence as an endemic in the Hawaiian Islands, presents some points of great interest. There is considerable doubt as to whether any of the dominant genes are identical with those of the other species. Identity of loci has, however, been demonstrated for nineteen factors. Two genes not found in any of the other species have been found, viz. **K^T** (khaki lint) and **H^T** (hairy plant body).

(4) *Darwinii* seems to be somewhat less widely separated from the other species than *tomentosum*. At least three entirely new genes were found, viz. **R^D** (red plant body), **Y^D** (yellow corolla) and **O^D** (laciniated leaf). The last gene falls into the multiple allele series for leafshape. It is probable that other dominant genes are specific alleles.

IX. HOMOLOGOUS LOCI IN NEW WORLD AND ASIATIC *GOSSYPIMUMS*

The New World species *G. barbadense* L. with $n=26$ -chromosomes crosses with some difficulty with *G. arboreum* L., one of the cultivated Asiatic cottons with $n=13$ -chromosomes. It has been shown (Harland, 1935 *a*) that by means of several back-crosses the initial great sterility of the hybrid was ultimately modified to complete fertility. In the *arboreum* genotype the gene **R** produced the character complex red plant body, red flower, and intense petal spot. When transferred to *hirsutum* the phenotypic expression of **R** was greatly weakened, producing a type with weak anthocyanin coloration of the plant body and flower and with *no petal spot*. It was considered that in the Asiatic group, **R** is accompanied by a constellation of modifiers, the combined effect of which is to enhance the manifestation of anthocyanin pigmentation, while the New World *G. hirsutum* either lacks such modifiers or carries neutralising modifiers in the other 13-chromosome subgenom.

The gene **R** already established to be a member of a multiple allele series of anthocyanin factors in the Asiatic group, proves to be also a member of a similar multiple allele series in the New World group. It is, however, a *new member* of the New World allele series, and this fact gives additional support to the theory that as we pass from one species to another we are likely to encounter new genes.

X. INTRODUCTION OF GENES FROM ONE SPECIES INTO ANOTHER.

The introduction of genes from one species into another provides evidence that species differ not only in the characteristic mode of distribution of alleles at the loci of "main genes" but also in the modifier complexes accompanying such alleles.

It is necessary at the outset to give some definition of a modifier and of a modifier complex. A modifier may be defined as a gene which affects the expression of another gene. It may have some effect on both members of a pair of alleles, or its effects may be confined to one member of the pair. Its function as a modifier may be purely secondary. Thus the factor pair S^B-s^B (presence and absence of petal spot) in *barbadense* is affected in its expression by the factor pair Y^B-y^B (yellow and cream corolla). $Y^B S^B$ is more intense than $y^B S^B$, while $Y^B s^B$ is a little stronger than $y^B s^B$. That is, Y^B acts as an intensifier of spot, with much effect on the dominant allele, and proportionally *less effect* on the recessive.

The factor H^B (*tomentosum* hairiness) acts as a modifier of leaf shape in *barbadense*, making the leaf lobes relatively shorter in proportion to their length.

Such modifying effects of single genes can be easily followed out. When, however, the expression of a character pair is conditioned by a pair of alleles on a given genetical background, and where changes in the genetical background assignable to an unknown number of genes result in corresponding changes in the phenotypic expression of the character pair, we must call the association of genes responsible for the change a "modifier complex". All dominant genes in *barbadense* seem to be accompanied by a complex composed presumably of tiny genes the combined effect of which is to enhance the expression of the main or primary gene. These can be termed "plus modifiers".

It is stated above that the two species *barbadense* and *hirsutum* differ in the modifier complexes accompanying what have been termed "main genes". Some examples will make this clear.

Investigations on petal spot.

In both *barbadense* and *hirsutum* there exist forms both with and without a large and intense purple spot at the base of the petal. The shape and intensity of the spot is not, however, quite the same in the two species, though the differences are not easily described.

In the cross *barbadense* spotted \times *barbadense* spotless (weakly spotted) the F_1 showed almost complete dominance of spot, and in F_2 a ratio of 3 spotted : 1 spotless (or weakly spotted). The dominant and recessive classes were easily separable. The cross *hirsutum* spotted \times *hirsutum* spotless behaved similarly with an even clearer type of segregation.

In *barbadense* (spotted) by *hirsutum* spotless these simple results were not obtained. The F_1 was spotted, but considerably less so than in *barbadense*. For the classification of the F_2 it was necessary to use twenty-two grades of spot, minutely graded from spotless to a type of spot more intense than that of the *barbadense* parent. The F_3 generation gave results for the most part as complicated. Sometimes the

3:1 ratio would seemingly occur, but cases of apparent dominance of spotless over spotted were also seen.

Nevertheless it proved possible to analyse this typical case of blending inheritance. The heterozygote was back-crossed several times to the *barbadense* spotted parent, with the result that clear monohybrid segregation was ultimately obtained on selfing, into 3 spotted:1 weak-spotted, indicating that *hirsutum* did carry an allelomorph of spotted. The characteristic blending obtained in F_2 was explained by the hypothesis that *barbadense* carried a gene for spot S^B but also carried an unknown number of modifiers, the combined effect of which with the recessive s^B produced a weak spot and with the dominant S^B a strong spot. *Hirsutum* spotless carried the allelomorph s^B of the spot gene S^B but few or none of the group of plus modifiers. In the interspecific cross blending resulted from the interaction of the factor pair S^B-s^B with a series of genotypical backgrounds produced by all possible spot modifier combinations. To sum up: the character "petal spot" in *barbadense* is due to a gene S^B which is accompanied by a modifier complex which strengthens the effect of the primary gene in that species. In *hirsutum* the spotless condition is due to the allele s^B combined with the alleles of many or most of the genes composing the spot modifier complex of *barbadense*.

The theory that *hirsutum* contains few or none of the *barbadense* plus modifiers of spot receives support from the results of experiments to transfer the *barbadense* spot gene S^B to a *hirsutum* genotype by the substitution of the *barbadense* modifiers for those of *hirsutum*. By repeatedly back-crossing heterozygous $S^B s^B$ plants to *hirsutum* it was found that the grade of spot became considerably weaker and ultimately gave a homozygous type of weakly spotted *hirsutum*. This could be regarded as the manifestation of the *barbadense* S^B gene, denuded or stripped of its plus modifiers by being placed on a *hirsutum* genotypic background. Appropriate experiments demonstrated that S^B and S^H were alleles at the same locus.

XI. RESULTS FROM TRANSFERENCE OF OTHER DOMINANT GENES FROM *BARBADENSE* TO *HIRSUTUM*.

Numerous experiments have been carried out in which dominant alleles have been transferred by repeated back-crossing from *barbadense* to *hirsutum*. Some typical results are put forward in Table III.

Table III. *Manifestation of barbadense dominant genes in hirsutum genotype.*

Gene pair	Effect in <i>barbadense</i>	Effect in <i>hirsutum</i>
$RB-rB$	Red plant body—green plant body	Weak red plant body—green plant body
$YB-yB$	Yellow corolla—cream corolla	Pale yellow corolla—almost white corolla
$P-p$	Strong yellow pollen—dark cream pollen	Pale yellow pollen—almost white pollen
S^B-s^B	Strong petal spot—weak petal spot	Weak petal spot—no petal spot
$KB-kB$	Khaki lint—cream lint	Pale khaki lint—white lint

Here we see that the phenotypic manifestation of five dominant *barbadense* genes is much weakened when transferred to the *hirsutum* genotype. It may there-

fore be argued that the presence of the dominant genes in *barbadense* is in some way connected with the presence of plus modifiers, and that a similar connection exists between the presence of the recessive allele and the *absence* of the modifier complexes. It may be further noted that the presence of the modifier complex or at any rate possibly of a considerable number of genes composing it can be noted by inspection, since the recessive characters in *barbadense* are characterised by an augmentation of the expression of the character. In some cases, indeed, a recessive allele accompanied by the whole plus modifier complex can exhibit more colour than the dominant allele stripped of its modifiers.

The weakening of a dominant character in an interspecific cross was noted by Gates (1915). He showed that the red of *Oenothera rubricalyx* became permanently diluted by crossing with *O. grandiflora*, and that the degree of dilution was increased every time the hybrid was again back-crossed to that species.

It has been established that in respect of "main genes", *hirsutum* and *barbadense* differ very markedly in that the species carry different dominant alleles; and this is possibly true also of recessives. Now the number of modifiers of the "main" genes must be enormously greater than the number of the main genes, and though some must act as modifiers of more than one primary gene it is clear that the number of genic differences between *hirsutum* and *barbadense* must be very great. The genetical formulae of the species of *Gossypium* given in the preceding section thus means very little from a taxonomic point of view, since what is important is the degree to which modifier complexes differ in the species.

Investigations on the crinkled dwarf mutant.

Although the experiments on the transference of dominants from *barbadense* to *hirsutum* have revealed much of interest, the experiments with the character known as "Crinkled Dwarf" have gone further to elucidate the nature and function of modifier complexes.

The crinkled dwarf mutant was first described (Harland, 1916, 1918) as a simple monohybrid recessive to normal in Sea Island (*G. barbadense* L.). Subsequently it has been twice recorded *de novo* in pure lines of Sea Island cotton both in Montserrat and St Vincent, and the wrinkled leaf mutant of Egyptian cotton recorded in Egypt has been proved by appropriate crosses to be identical with it. Since it is not known to occur in *G. hirsutum* L. and provides an example of what may be termed a taxonomically delimited mutant, its behaviour in crosses with that species has been followed in great detail. By means of ten back-crosses the crinkled dwarf mutant has been introduced into three different varieties of *hirsutum*, and the summarised results of the experiments with crinkled will be found in Table IV.

The following conclusions may be drawn from these experiments:

(1) The normal allele of crinkled in *barbadense* and *hirsutum* (T 57) is accompanied by a modifier complex consisting of a number of genes the combined effect of which is to render "normal" dominant over crinkled. The special case of T 9 will be discussed later.

Table IV.

A. Crosses of *barbadense* crinkled \times *barbadense* and *hirsutum* normals.

Exp.	Cross		F_1	F_2
	Crinkled parent	Normal parent		
1	<i>b.</i> (Sea Island)	<i>b.</i> (Sea Island)	Normal	3 Normal : 1 crinkled
2	<i>b.</i> (Egyptian)	<i>b.</i> (Sea Island)	Very faintly crinkled	3 Normal : 1 crinkled (some normals with faint crinkling as F_1 but segregation clear)
3	<i>b.</i> (Sea Island)	<i>h.</i> T 9	Intermediate crinkled	Series of graded types from exaggerated (super) crinkled to normal
4	<i>b.</i> (Sea Island)	<i>h.</i> T 57	Faintly crinkled	Do.

B. Crosses of homozygous *hirsutum* crinkleds (produced by repeated back-crossing of heterozygotes to Normal *hirsutum*) \times various Normals.

Exp.	Cross		F_1	F_2
	Crinkled parent	Normal parent		
5	<i>h.</i> T 9	<i>b.</i> (Sea Island)	Normal	Series of unclassifiable types ranging from super-crinkled to normal
6	<i>h.</i> T 57	<i>b.</i> (Sea Island)	Normal	Do.
7	<i>h.</i> T 9	<i>h.</i> T 9	Intermediate	1 Normal : 2 Intermediate : 1 crinkled
8	<i>h.</i> T 9	<i>h.</i> T 57	Very faint crinkling	3 Normal : 1 crinkled (some normals with faint crinkling)
9	<i>h.</i> T 57	<i>h.</i> T 57	Normal	3 Normal : 1 crinkled

C. Crosses between extracted *hirsutum* crinkleds and other types of crinkled.

Exp.	Crinkled parents	F_1	F_2
10	<i>h.</i> T 9 \times <i>b.</i>	Crinkled	Range from super-crinkled to apparently normal
11	<i>h.</i> T 57 \times <i>b.</i>	Crinkled	Do.
12	<i>h.</i> T 9 \times <i>h.</i> T 57	Crinkled	All crinkled but considerable variation from good to bad

b., *barbadense*; *h.*, *hirsutum*.

(2) The genes constituting the modifier complexes affecting the manifestation of the crinkled gene are different in the two species and consequently the F_2 of (1) *hirsutum* normal \times *barbadense* crinkled, (2) *barbadense* normal \times *hirsutum* crinkled or (3) *hirsutum* crinkled \times *barbadense* crinkled exhibits blending of an extreme kind. The reasons for the type of blending will be easily understood when it is realised that homozygous crinkled on some genetical backgrounds is pseudo-normal and heterozygous crinkled on other backgrounds is phenotypically crinkled.

(3) The experiments indicate that *barbadense* and *hirsutum* possess, in respect of crinkled modifiers, what can be termed a different genetical architecture, and from extensive but uncompleted experiments on the behaviour of other genes in the interspecific cross we are inevitably led to the conclusion that the two species are built up on an altogether different plan in respect of all their modifier complexes.

(4) If the genetical architecture of the two species is different and if, as has been abundantly demonstrated, characters are constructed on a different plan in the two species, we may obtain some knowledge of the nature of that plan by crossing, since either species will "fractionate" the other in so far as the modifier complexes differ in genetic content.

Are the Normal alleles of Crinkled the same in barbadense and hirsutum?

So far it has been shown that the modifier complexes affecting the expression of crinkled are different in *barbadense* and *hirsutum*, and that these modifier complexes tend to be somewhat different in such closely allied forms as Egyptian and Sea Island and in the two *hirsutum* forms T 9 and T 57.

We may now ask whether the Normal alleles of crinkled are alike in *barbadense* and *hirsutum*. Using the three homozygous types of crinkled (*barbadense* (Sea Island) crinkled, *hirsutum* T 9 crinkled, and *hirsutum* T 57 crinkled) as bases, it is possible to transfer the various Normals on to them by repeated back-crosses. The results of these experiments are summarised in Table V.

Table V. Results of transference of various Normals to Crinkled bases.

Type of Crinkled base	Type of Normal	Phenotype of heterozygote
<i>barbadense</i> (Sea Island)	T 8 (<i>hirsutum</i>)	Intermediate
Do.	T 57 (<i>hirsutum</i>)	Very slightly crinkled
Do.	T 18 (<i>barbadense</i>)	Normal
<i>hirsutum</i>		
T 9	T 18 (<i>barbadense</i>)	Normal
Do.	T 57 (<i>hirsutum</i>)	Very slight
Do.	T 9 (<i>hirsutum</i>)	Intermediate
<i>hirsutum</i>		
T 57	T 18 (<i>barbadense</i>)	Normal
Do.	T 57 (<i>hirsutum</i>)	Normal

It will be seen from the above results that there is good evidence of three different Normal alleles of the crinkled mutant (c^R). The *barbadense* allele exhibits complete dominance on all three crinkled backgrounds. The *hirsutum* T 57 allele shows complete dominance on its own crinkled background, incomplete on crinkled *barbadense*, and nearly complete on crinkled T 9 *hirsutum*. The *hirsutum* T 9 allele exhibits incomplete dominance on its own crinkled background, and also on the *barbadense* crinkled base.

To sum up: it has been shown that *barbadense* possesses one allele of Crinkled which may be termed C^{RB} , and that *hirsutum* has two more different alleles, namely C^{RM} (in *hirsutum* var. Meade T 9) and C^{RH} (in *hirsutum* var. Triumph T 57). These three alleles are distinguishable only by their dominance reactions on various crinkled genetical backgrounds, and from uncompleted experiments it is already clear that further different normal alleles are present in the other four species.

XII. EXPERIMENTS WITH CRINKLED AND FISHER'S THEORY OF DOMINANCE.

In a series of papers Fisher (1928*a*, 1929, 1931) has brought forward a new theory of dominance. A brief summary of his theory may be given.

The appearance *de novo* of a mutant has usually been observed in a heterozygote, which on being inbred produces 1 wild type or normal : 2 heterozygotes : 1 mutant. Although mutants occur rarely in laboratory experience, they must have occurred many times in the evolutionary history of the species, and as heterozygotes must therefore have been exposed to the play of natural selection. Since mutants are usually at a disadvantage compared with the wild type, and since even one dose of a deleterious gene would probably produce some disadvantageous effect, selection of the most favoured heterozygotes would result in the preservation of those possessing the greatest degree of dominance over the mutant. Dominance over a mutant is then, according to Fisher, the result of an evolutionary process by which an initially intermediate and disadvantageous heterozygote accumulates favourable modifying genes which cause it ultimately to resemble the wild type both in phenotypical appearance and in physiological characteristics. Continuous interplay between the heterozygote and modifiers must be regarded as an essential feature of the theory.

The experiments with "Crinkled" have been cited by Fisher (1931) in support of his theory, but the final results seem to disprove the main hypothesis contained therein. The bearing of the experiments on the Fisher theory may therefore be discussed in detail.

In the first place the genetical architecture of the dominance reaction systems of *hirsutum* and *barbadense* in regard to the mutant Crinkled Dwarf have been shown to differ as follows:

Species	Normal allele	Modifier system	Dominance reaction
<i>barbadense</i> (T 18)	C ^{RB}	Specifically <i>barbadense</i>	Complete dominance
<i>hirsutum</i> (T 9)	C ^{RM}	Probably mixture of <i>barbadense</i> and <i>hirsutum</i> genes	Incomplete dominance
<i>hirsutum</i> (T 57)	C ^{RH}	Specifically <i>hirsutum</i>	Complete dominance

The modifier complex of *hirsutum* (T 9) has also been shown to differ from that of *hirsutum* (T 57), since a range of crinkled phenotypes occurs in F_2 of the cross between them, and the explanation previously given (1933) of the failure of dominance in T 9—that T 9 is not a pure *hirsutum* but has had its dominance reaction system impaired by *barbadense* genes—is thus confirmed by further work.

Now if two specific sets of dominance modifiers are brought into contact, as in the *barbadense-hirsutum* cross, there must occur in F_2 a mutual breakdown of the dominance mechanisms, since each species will presumably contain alleles of the dominance modifiers of the other. The isolation from the interspecific cross of a new type of exaggerated crinkled (super-crinkled) upon which C^{RM}, C^{RH} and C^{RB} show some failure of dominance—most with C^{RM}, less with C^{RH} and very slight

with C^{RB} —indicates that the nature of the modifier complex is as important in deciding the dominance reaction as the nature of the normal allele.

The normal alleles themselves can, however, be arranged in order of dominance potency, and since it is clear that on a T₉ background the mere substitution of C^{RB} for C^{RM} converts incomplete into complete dominance, and since a condition of complete dominance also exists in a species where the mutant has never occurred, we must assume that the attainment in a species of dominance over a deleterious mutation may be quite unconnected with modification of the reaction of the species to the mutant through the occurrence of initially disadvantageous heterozygotes. Consequently the weight of evidence is against the Fisher theory as first put forward, since the repeated occurrence of heterozygotes would appear to be an essential feature of the theory.

Our final view is that the modifier systems of the genes affecting dominance have been *selected on their own account* because the complex as a whole is of advantage, that the genetical architecture of the modifier systems of *barbadense* differs profoundly from that in *hirsutum*, and that the degree of potency of the normal allele is an important factor in deciding whether the whole reaction system shall exhibit complete dominance.

Here it is important to note and adopt the suggestion of Haldane (1930) that plus mutations at any locus may be favoured by selection provided that the original gene is not completely dominant, since the original type of gene is at a disadvantage and the new gene is not. Fisher (1931) accepted this as supplementing the mechanism of dominance evolution which he first suggested, namely, the spread of modifying factors which increase dominance. Haldane further says (1933): "It is far from certain which of the two secondary effects is likely to have the greatest effect in increasing dominance." It is clear that changes both in normal alleles (the Haldane effect) and in modifiers have both taken place in *Gossypium*, and the occurrence of the normal allele C^{RB} , with the strongest dominance potency in the species where the mutant is known to occur, would argue that the weaker alleles have been selected against and probably eliminated in *barbadense* as a consequence of the occurrence of the crinkled mutant.

The discovery of weaker normal alleles in *hirsutum* strengthens this point of view.

It is interesting to note the statement made by Haldane (1927) in a survey of the comparative genetics of colour in rodents and carnivora that "The data for the species under consideration suggest that evolution has occurred, to some extent at least, by slight changes in the intensity of action of genes".

XIII. HOMOLOGOUS CHARACTERS EXISTING THROUGHOUT THE GENUS, WHICH MAY BE GENETICALLY CONSTRUCTED IN DIFFERENT WAYS.

The existence of homologous characters throughout a genus, or even throughout a series of related genera, has been discussed in detail by Vavilov (1922), but few or no observations have been up to the present available on the genetics of homologous characters in related species.

The six species of New World cottons and the eleven 13-chromosome species possess a series of such homologous characters as red and green plant body, yellow and cream pollen, yellow and cream corolla, brown and white lint, presence and absence of seed fuzz.

There are three ways in which homologous characters can be constructed in the six New World *Gossypium*s:

(1) By using different alleles of a multiple allelomorph series, together with a specific modifier complex.

The two cases below are representative of what seems to be a rather general phenomenon, though the data regarding other characters are less complete.

Character	<i>hirsutum</i>		<i>barbadense</i>	
	Main gene	Modifier complex	Main gene	Modifier complex
Normal v. Crinkled Spot v. spotless or faintly spotted	CRM or CRH SH	Specifically <i>hirsutum</i> Do.	CRB SB	Specifically <i>barbadense</i> Do.

In these two cases the character is constructed by a gene complex, the only common resemblance being that the main or key gene is at the same locus in the two species.

(2) By employment of different members of a pair of duplicate genes, in association with a specific modifier complex.

Character	<i>hirsutum</i>		<i>barbadense</i>	
	Main gene	Modifier complex	Main gene	Modifier complex
Khaki lint—white or pale brown lint	KH	Specifically <i>hirsutum</i>	KB	Specifically <i>barbadense</i>
Red plant body—green plant body	RH	Do.	RB	Do.
Green—chlorophyll deficient	Chb	Do.	Cha	Do.

(3) By accumulation of plus modifiers in absence of the main gene.

Two examples of this process will suffice.

A cross between *barbadense* weak spotted by *hirsutum* spotless gives F_1 very faintly spotted, and in F_2 a range from spotless to fairly strongly spotted. The weakly spotted *barbadense* is thus further modified in a plus direction by modifiers from *hirsutum*. The latter thus act as modifiers of modifiers (cf. Ibsen, 1932).

Recombination of crinkled modifiers in *barbadense* crinkled \times *hirsutum* crinkled results in a new type of normal (pseudo-normal) which bred true for seven generations.

There is considerable reason for the belief that duplicate factors may often be a consequence of polyploidy, and as previously pointed out the investigations of Skovsted (1934) show that the New World species of *Gossypium* are most probably allopolyploids.

Whether duplication of factors in the tetraploid *Gossypium*s took place through polyploidy cannot be said with certainty, and it is probable that duplication occurred merely of loci carrying different species alleles.

Haldane (1932) mentions the fact that allopolyploids "possess several pairs of sets of genes, so that one gene may be altered without disadvantage provided its functions can be performed by a gene in one of the other sets of chromosomes", and further (1933), "The final result will be that most of the genes will return to the diploid condition". The mode of distribution of the two chlorophyll-deficient genes c^{ha} and c^{hb} in the six New World species (Harland, 1934 *b*) provided what appears to be the first experimental evidence bearing on this theory. Here the position is summarised as follows: "If as a consequence of polyploidy a large number of genes become duplicated, and the characters governed by such genes are of importance to the species, one of the members may mutate, leaving the character unimpaired, with the further possibility that the mutation may be of benefit to the species.

"The duplication of all the genes in an autopolyploid should mean that a large number, possibly several hundred genes are liberated for mutative purposes with concomitant augmentation of genetic variance and potentiality for evolution."¹

The provision of even a partial explanation of the Vavilov law of homologous variation is of great interest, and the conception that in two species the genetical architecture of the same character or organ becomes widely different is probably of profound evolutionary significance.

As I have previously said (1933): "We have a clear indication that a character or organ is not genetically in a static but is in a dynamic condition. The genes, as a manifestation of which the character develops must be continually changing, according to whether their allelomorphs are selected to strengthen other physiological processes. There must be continual competition between different organs or functions for one or the other member of a pair of allelomorphs.

"On the dynamic view of organs and functions we are able to see how organs such as the eye, which are common to all vertebrate animals preserve their essential similarity in structure or function, though the genes responsible for the organ must have become wholly altered during the evolutionary process, since there is now no reason to suppose that homologous organs have anything genetically in common.

"The dynamic conception of the evolution of function serves also to explain the remarkable results obtained by Goldschmidt (1933) in his crosses between European and Japanese races of the gypsy moth (*Lymantria dispar*). Goldschmidt obtained intersexes in these crosses, but explains the results on a theory involving the assumption of quantitative potencies of the sex genes."

It is clear that as a consequence of geographical isolation and different ecological environment, the whole sex mechanism of *Lymantria* has become built up differently in the two widely separated areas, and that on crossing there occurs mutual dis-

¹ Mr R. A. Silow informs me that he has studied the inheritance of petal spot in two species with $n=13$ chromosomes, viz. *G. arboreum* and *G. anomalum*. Petal spot is determined in these two species by main genes at different loci. This case however, although probably not strictly one of duplicate genes, indicates that characters may be constructed in genetically different ways even in the diploid section of the genus *Gossypium*.

integration of the two sex mechanisms leading to the production of intersexes, but the evidence is not conclusive as to whether variations in dominance potency of main genes or changes in modifier complexes have been more important.

It seems evident that if Goldschmidt's assumption of a series of alleles or of complexes behaving as such is accepted, we must also accept the assumption that the modifier complexes have changed so as to permit the substitution of new alleles with different properties *pari passu* with the change in sex balance. The *Lymantria* situation thus closely parallels that found with Crinkled Dwarf in *Gossypium*.

XIV. GENERAL CONSIDERATIONS.

Evidence has been brought forward that in the two species *Gossypium barbadense* and *G. hirsutum*, long separation has produced profound genetic changes. This is true of four other species, so far as they have been examined. Many genes have evolved into new alleles, homologous characters have become genetically differently constructed, and new co-ordinated modifier systems set up. There is nothing in the experiments to indicate that any other process except gene substitution has taken part, and nothing to indicate that the Darwinian process of gradual evolution by natural selection has not been essentially the main mechanism involved in the great changes which have taken place.

It may here be stated that the statement made above that new co-ordinated modifier complexes have arisen in *Gossypium* is essentially in harmony with the view expressed by Timoféeff-Ressovsky (1932 *a*) in his preliminary account of the geographical races of *Epilachna chrysomelina*. He concludes that the geographical races in this species differ from each other in complexes of genes, and that not single genes, but rather "harmonic" gene combinations have selective values under a given set of geographical conditions.

Cytological considerations have been purposely omitted, since this review was meant to deal only with genic changes. It is clear, however, that changes in the number and method of construction of chromosomes have been of great importance in other genera, and it is not possible to predict how far the processes demonstrated for *Gossypium* are of wide occurrence.

A survey of the morphological and physiological characteristics of the six species of *Gossypium* under consideration reveals the important point that changes in structural characters have been far less important than changes in physiology. The differences between *G. tomentosum*, an extreme xerophyte, and the other species may rather be a matter of hundreds or possibly thousands of minute "physiological" genes. A cross between *tomentosum* and *barbadense* gives an F_1 intermediate in physiological reaction to the extremely humid climate of Trinidad. The back-cross to *barbadense* behaves in a similar way to pure *barbadense*, whereas the back-cross to *tomentosum* behaves practically like *tomentosum*, i.e. adaptive behaviour is directly proportional to the number of *tomentosum* or *barbadense* genes present.

Here it is well to recall the view of Baur (1924) that tiny mutations are extraordinarily frequent in *Antirrhinum*. These affect all possible morphological and physiological characters. They do not condition any monstrous or pathological

alteration but produce changes still fully within the normal. These small mutations, he maintains, manifestly play a very important role in evolution.

The experimental evidence summarised in the present review is not only in entire accord with the earlier views of Baur, and the recent writings of Fisher, Haldane and others, in support of the Darwinian theory of gradual evolution through natural selection, but should go some way to lessen the wide gap between the views on evolution of the taxonomist or paleontologist and those which they have supposed the geneticist to hold.

To what extent do the six species of New World cottons under discussion possess genes in common? It is known that alleles at the same locus are held in common, and it is probable that certain common recessives such as *y* (cream corolla) are identical. If, however, the genetical architecture of *hirsutum* and *barbadense* were completely different in respect of complex morphological entities such as a flower, we should expect more or less mutual disintegration of the whole genetical complex and consequently many abnormalities in the morphology and developmental physiology of the flower. Actually in this and other interspecific crosses certain flower abnormalities are fairly frequent and may be ascribed to the lack of harmony between the two gene systems for flower formation in the species cross. Such features as extra petals or stigmas completely enclosed by the staminal sheath are among the common abnormalities. The fact that most combinations possess perfect flowers would, however, argue that the species possess either a common stock of identical genes or an assemblage of mutually replaceable ones.

It has been pointed out by Baur (1932) that in the F_2 of interspecific crosses in *Antirrhinum* a large number of weak, relatively sterile forms occur which are ill-adapted for existence. This phenomenon is extremely prevalent also in *Gossypium*, and the explanation obviously lies in the mutual disintegration by crossing of the specific harmonic associations of genes which build up vital physiological mechanisms according to different plans.

It is clear that the existence of variations in genetical architecture in the main subgroups of man himself would produce disharmonic combinations in such wide crosses as Oriental-European or Lapp-Swede (Mjøen, 1931). A susceptibility to tuberculosis and other disorders in F_2 over and above that possessed by the racial stocks of the parents or of the F_1 would be expected on the hypotheses presented in this paper, and there seems no reasonable doubt that it occurs. The importance of knowledge of this kind to the whole science of eugenics cannot be over-estimated.

XV. SUMMARY.

1. Previous work on the behaviour of genes in interspecific crosses is discussed, and it is concluded that allelomorphous relationships exist between the genes of crossable species whether these are in the same genus or in reputedly different genera.

2. Genetical experiments on interspecific hybrids in six species of the genus *Gossypium* enable the following main conclusions to be drawn:

(a) Although allelomorphous relationships exist throughout the six species in respect of all genes examined, cases of identity (apparent or real) are practically

confined to recessive genes only. Geographical isolation over a long period of time has resulted in the production of new alleles at most loci. These may be termed "species alleles". Species endemic in the Galapagos and Hawaiian Islands are characterised by species alleles not found in mainland species.

(b) The introduction of genes from one species into another indicates that species differ not only in the mode of distribution of alleles functioning as main genes, but also in the modifier complexes accompanying such alleles. The degree to which modifier complexes differ in species is of primary importance from a taxonomic point of view.

(c) The bearing of experiments with the "Crinkled" mutant on the Fisher theory of dominance is discussed. It is shown that a number of normal alleles of crinkled exist, which are distinguishable only by their dominance potency, that the dominance relation is due to the interaction of a normal allele of specific potency with a modifier complex to which it is precisely adjusted, and that the genes constituting the dominance modifier complex have been preserved not because of their function as modifiers of initially disadvantageous heterozygotes, but because of their selective value on their own account. It is believed, however, that on the appearance of the crinkled mutant, selection probably ensued in favour of alleles with greater dominance potency, *i.e.* that the "Haldane effect" has been operative.

(d) Examples are given of three different ways in which homologous characters can be genetically constructed in the genus *Gossypium*. It is pointed out that the conception of continuous change with time in the genetical architecture of an organ is probably of profound evolutionary significance.

3. The Darwinian process of evolution by natural selection, involving mere gene substitution, has probably been the main mechanism involved in the profound changes induced in the genus *Gossypium* through geographical isolation.

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THE EQUILIBRIUM FUNCTION OF THE VERTEBRATE LABYRINTH

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I. INTRODUCTION.

THE classical theory of the equilibrium function of the labyrinth, developed by Mach, Breuer and Brown during the last quarter of the nineteenth century, has been the centre around which the whole structure of subsequent knowledge and theory has been built up for half a century. It is still the basis of text-book descriptions and reviews of the subject.

In recent years, however, experimental results have been obtained from all vertebrate classes except reptiles, which show that this theory is at fault at some important points. The present article is a survey of such of these results as have not previously been reviewed in detail.

II. MORPHOLOGY AND STRUCTURAL AND FUNCTIONAL SUBDIVISION OF THE LABYRINTH.

Fig. 1 is a diagram combining the different characters of the membranous labyrinth in all vertebrate classes. There are usually three semicircular canals, two

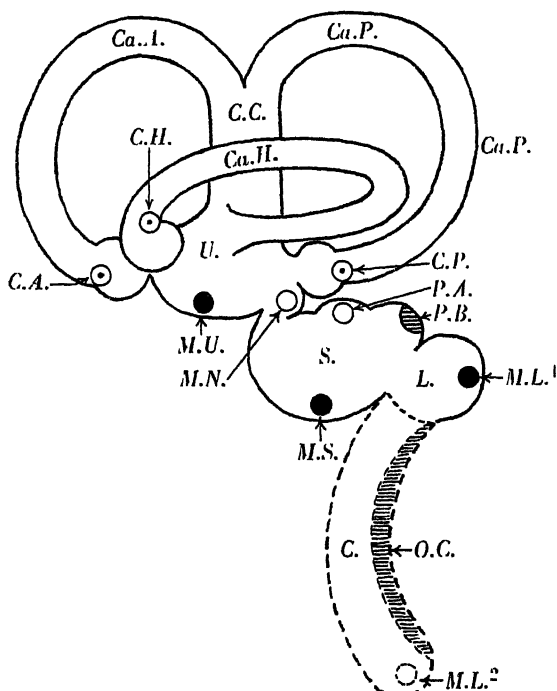


Fig. 1. Diagrammatic combination of the characters of the membranous labyrinth in all vertebrate classes. *C.*=cochlea; *C.A.*=crista anterior; *C.H.*=crista horizontalis; *C.P.*=crista posterior; *C.C.*=crus commune; *Ca.A.*=canalis anterior; *Ca.H.*=canalis horizontalis; *Ca.P.*=canalis posterior; *L.*=lagena; *M.L.*¹=macula lagenae in fishes and amphibia; *M.L.*²=macula lagenae in reptiles and birds; *M.N.*=macula neglecta=papilla neglecta; *M.S.*=macula sacculi; *M.U.*=macula utriculi; *O.C.*=organ of Corti; *P.A.*=papilla amphibiorum; *P.B.*=papilla basilaris; *S.*=sacculus; *U.*=utriculus. The ductus endolymphaticus is omitted for the sake of clarity.

vertical canals and, except in cyclostomes, a horizontal one. The semicircular canals lie in planes approximately perpendicular to each other. Each canal widens at one end into an ampulla containing a sense ending called the crista ampullaris, consisting of secondary sense cells. The crista bears as an auxiliary structure a jelly-like cupula which encloses the sense hairs protruding from the surface of the crista (Fig. 2). Three sac-like cavities, the utricle, the saccule and the lagena, contain sense endings which are called maculae. The sense cells forming the maculae are similar

to those of the cristae, differing from them only by having shorter sense hairs. The sense hairs are embedded in an otolith membrane covering the macula. These membranes are encrusted with lime which either forms solid stones (the otoliths of bony fishes) or numerous concretions of lime crystals (otoconia) (Fig. 2). In myxinoids (cyclostomes) only one macula, the macula communis, is present. The macula lagenae is transferred to the distal end of the cochlea in birds, and with a few exceptions is lacking in mammals. The cochlea, the first trace of which is to be found in the amphibians as a papilla basilaris, reaches its highest development in mammals. It contains the organ of Corti.

Two further sense endings occurring in the vertebrate labyrinth are the papilla neglecta and the papilla amphibiorum which have often been confused with one

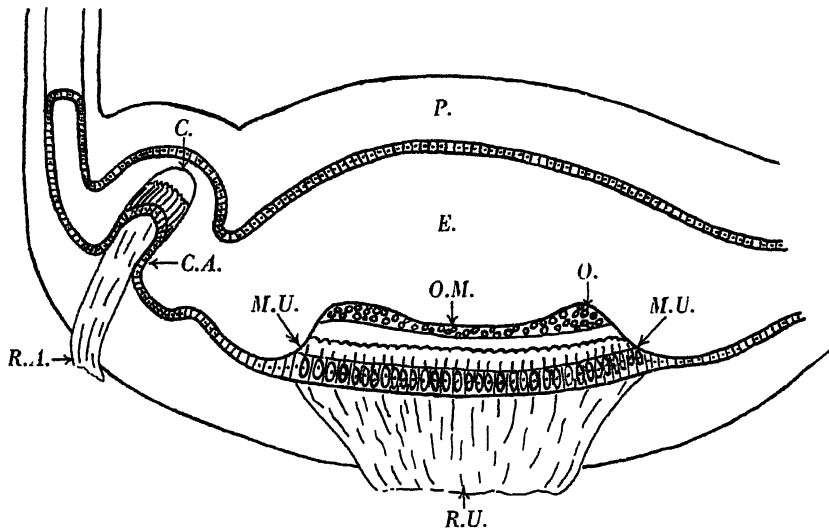


Fig. 2. Section through the macula utriculi and the ampulla of a vertical semicircular canal in man (after Kolmer). C.=cupula; C.A.=crista ampullaris; E.=endolymph; M.U.=macula utriculi; O.=otoconia; O.M.=otolith membrane; P.=perilymph; R.A.=ramus ampullaris; R.U.=ramus utricularis of the nervus vestibularis.

another. According to de Burlet (1928) the papilla neglecta is a sense ending belonging to the utriculus. It occurs in representatives of all vertebrate classes but is very often missing in mammals and amphibians. Its covering auxiliary structure is similar to a cupula or otolith membrane. The papilla amphibiorum only occurs in amphibians. Its auxiliary structure is similar to the membrana tectoria of the organ of Corti.

The membranous labyrinth, the cavities of which are filled with endolymph, is surrounded by cartilage or bone, the space between it and the surrounding tissues being filled with perilymph.

The labyrinth is innervated by the 8th nerve which splits up into several branches going to different parts of the organ.

After Flourens had found in 1824 that the cochlea, with its organ of Corti, is

concerned in hearing and had performed the first experiments on the semicircular canals of the pigeon, showing their equilibrium function, the labyrinth was regarded as divided into an acoustic part represented by the cochlea and a so-called static part represented by all the remaining sense endings. This static part was subdivided again into the semicircular canals and the otolith organs.

This subdivision formed the basis for the classical theory of the equilibrium function of the labyrinth evolved almost simultaneously by Mach, Breuer, Brown, and the later researches of Ewald, Magnus and de Kleijn, Quix, de No, and Wittmaak were theoretically based upon this subdivision.

A second subdivision, which has recently become more and more important, is topographical in origin. The labyrinth is in this case divided into a superior and an inferior part (*pars superior* and *pars inferior*). To the *pars superior* belong the semicircular canals and the utricle; the saccule and its various appendages form the *pars inferior*. De Burlet (1931) is right, however, in pointing out that this division into superior and inferior parts is topographically correct in higher vertebrates only. In cyclostomes and fishes, for example, the two parts are very often side by side and not one above the other. A common feature, however, is that in all vertebrates these two divisions of the labyrinth are more or less separated from each other. There is in many cases only a small membranous communication between them, the *canalis utriculo-saccularis*, and sometimes the separation is a complete one. This morphological arrangement has made possible operative separation of the two parts, and from a series of cases it is clear that this subdivision has not only a morphological significance but also a functional one.

A further subdivision arises from the fact that the 8th nerve divides into two main branches, the *ramus anterior* and the *ramus posterior*. In all vertebrates the macula utriculi, and the anterior vertical and the horizontal canals are innervated by the *ramus anterior*. The *ramus posterior* always supplies the crista of the posterior vertical canal, the macula lagenae, the papilla basilaris or the cochlea, and, when present, the papilla neglecta. The macula sacculi is innervated by a branch of the *ramus anterior*, by one or more separate nerve branches, by a branch of the *ramus posterior*, or by a combination of these three. According to de Burlet (1929) this inconstancy of innervation in the saccule can be explained by an equally great variation of the topographical position of the saccule in relation to the other parts of the labyrinth.

To the subdivisions just described de Burlet adds a fourth one, which is derived from the phylogenetic development of the labyrinth. According to this theory, the labyrinth originally consisted of symmetrical anterior and posterior halves (*Myxine*). In the other five classes of vertebrates, however, the labyrinth is asymmetrical antero-posteriorly. For a series of vertebrates de Burlet worked out the relations of the sense endings to the phylogenetically anterior and posterior halves of the labyrinth. The result was an almost complete coincidence of this subdivision with the one based on innervation. De Burlet therefore describes the innervation as being conservative in principle. It preserves its original morphological relations and thus gives evidence of phylogenetic development.

At the present time there is no experimental evidence that the two methods of subdivision last described are of any functional significance.

III. FUNCTION OF THE LABYRINTH AS A WHOLE.

Four different functions of the labyrinth are so far known. The labyrinth is:

- (1) An organ concerned in the maintenance and regulation of muscle tone.
- (2) A gravity receptor.
- (3) A receptor for linear and angular accelerations.
- (4) A sound receptor.

This follows from the fact that bilateral total labyrinth extirpation is followed by temporary or lasting loss in muscle tone, and in blinded animals by severe disturbances of balance and by deafness.

(1) TONUS.

Ewald (1892) was the first to show the effect of labyrinth eliminations on muscle tone. He evolved the theory of the "Tonuslabyrinth" and showed that loss in labyrinth tone is observed not only in muscles involved in balance and posture but also in muscles or muscle groups which do not seem to be under constant reflex control of the labyrinth, such as muscles involved in deglutition and mastication (experiments on dogs).

That tonic effect of the labyrinth which may extend over the whole of the nerve-muscular system has been described as a "stimulatory function" of the labyrinth (Wolsky, 1933). A stimulatory function is attributed to those sense organs which constantly send stimuli to the nervous muscular system, stimuli which do not result in specific reflex reactions but throw the muscles into a state of preparedness for action. A dominating stimulatory effect is ascribed to the labyrinth, while other sense organs, such as the eyes, the lower sense organs and the proprioceptors, may also be stimulatory in function. But Buddenbrock (1928), and in accordance with him Wolsky (1933), quite rightly point out that the stimulatory function of the labyrinth is only a supplementary one accompanying its main function, namely that of setting up the equilibrium reflexes. A clear distinction between the two functions is, however, just as difficult to make in the labyrinth as it is in the other sense organs mentioned above. For instance, the stimulatory tonic effect of the labyrinth must not be confused with the superposed tonic labyrinthine reflexes which will be dealt with in the next section.

The well-known forced poses of the head, the eyes, the extremities, and the body, following unilateral labyrinth extirpation in vertebrates, have been reviewed in detail amongst others by Maxwell (1923), Fischer (1926, 1930), Magnus (1924) and Magnus and de Kleijn (1926*a*, 1930). They may at least partly be attributed to asymmetrical tonus deficiencies of a general nature (Wolsky, 1933). Again, it is difficult to decide what part is played in these forced effector poses by asymmetric deficiencies in stimulatory muscle tone and what part by reflex deficiencies.

(2) LABYRINTHINE REFLEXES.

It is not within the scope of this review to deal in detail with the different types of labyrinthine reflexes. They too have been excellently reviewed for all vertebrate classes by Maxwell (1923), Fischer (1926), Magnus (1924), Magnus and de Kleijn (1926*a*), Camis and Creed (1930), and de No (1931). A short survey will therefore be sufficient.

(a) *Static reflexes.*

Static reflexes are compensatory poses of the eyes, head, extremities and trunk, which are evoked when the spatial orientation of an animal in relation to gravity

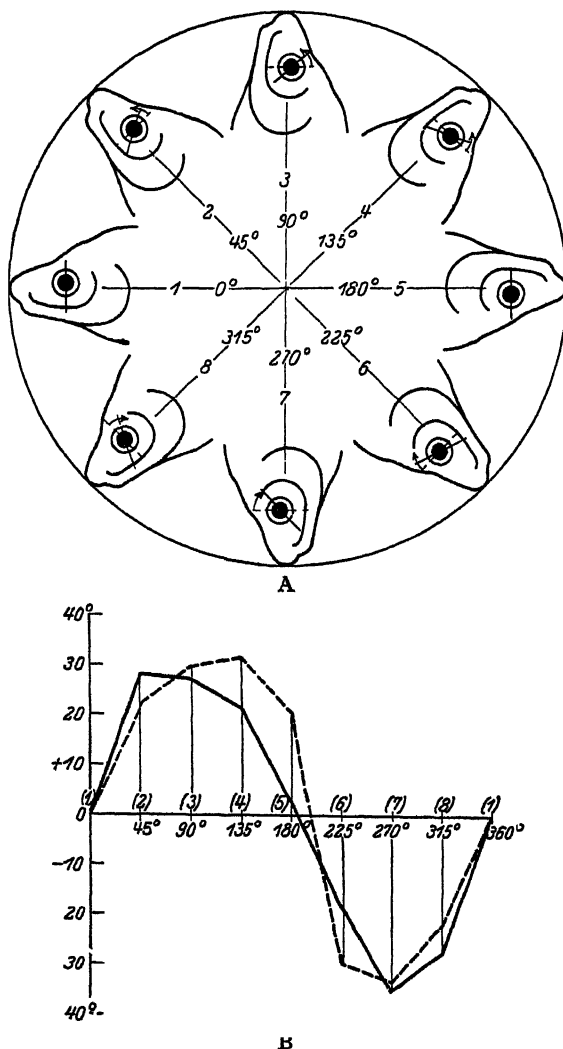


Fig. 3. A, rotatory eye deviations in the carp during changes of position around the animal's horizontal transverse axis. B, degree of eye deviation from the normal position of the carp (-----) and perch (——). + sign, forward rolling, - sign, backward rolling of the eye. (After Benjamins from Fischer (1926).)

differs from its normal spatial orientation. They are due to co-ordinated alterations in the tonus of the effector muscles and persist as long as the evoking spatial orientation is maintained.

(1) *Compensatory eye poses.* (a)¹ Rotatory deviation (Fig. 3). (b)¹ Vertical deviation (Fig. 4). These eye poses are compensatory because in a changed orienta-

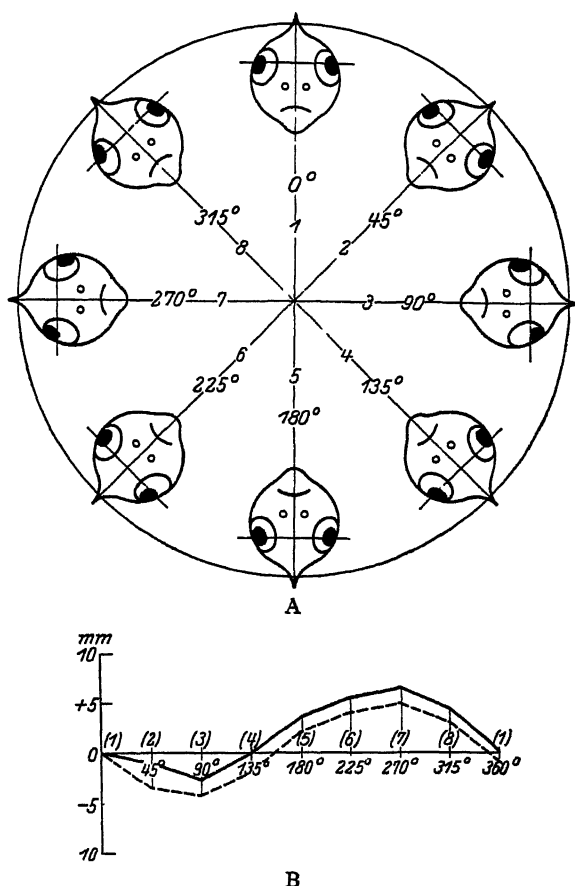


Fig. 4. A, vertical eye deviations in the carp during changes of position around the animal's horizontal longitudinal axis. B, degree of eye deviation from the normal position in the carp (-----) and perch (———). + sign, dorsal deviation, - sign, ventral deviation of the right eye (in mm.). (Modified after Benjamins from Fischer (1926).)

tion of the animal to gravity they maintain a visual field not very different from that seen in the normal orientation.

(2) *Compensatory head poses.* Animals which can move the head show compensatory head poses which are the result of labyrinth reflexes acting on the neck muscles (labyrinthine righting reflexes). The effect of these head poses is the maintenance of a horizontal orientation of the head in space when the orientation

¹ In animals with frontal eyes the vertical deviation replaces the rotatory one, and *vice versa*.

of the body to gravity is changed (Fig. 5), and further, the initiation of a chain of proprioceptive reflexes from the neck muscles acting on the limbs and the trunk, which finally lead to a resumption of the normal body orientation in space. The

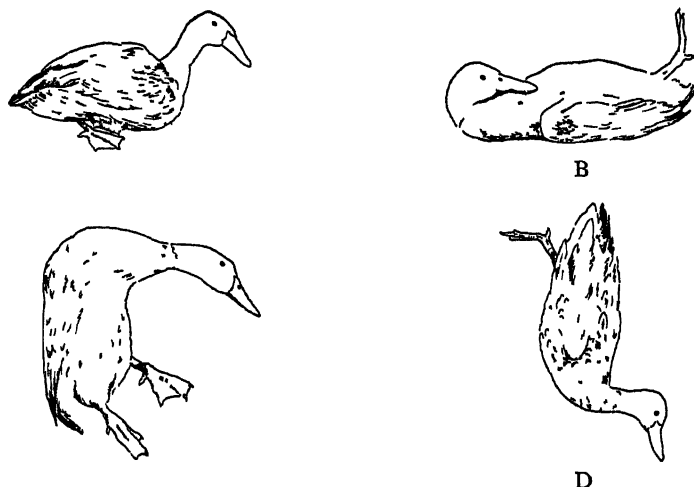


Fig. 5. Compensatory head poses in the duck: A=normal, B=supine, C=anterior end up, D=anterior end down. (After F. M. Huxley from Fischer (1926))

complicated interaction of labyrinthine and proprioceptive reflexes in mammals has been analysed by the Utrecht school of physiologists under Magnus. A detailed description may be found in Magnus, *Körperstellung* (1924). No such profound analysis has yet been carried out in other vertebrates.

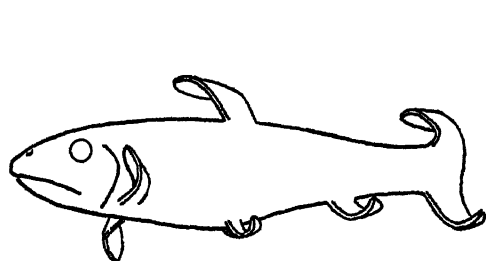


Fig. 6. Compensatory fin poses in a fish when turned on to its right side.

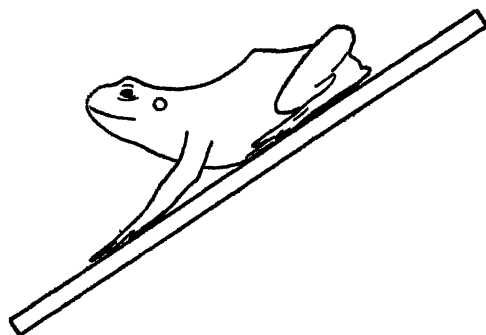


Fig. 7. Compensatory stretching of the fore-limbs of a frog when sitting on a downward slope. (Modified after Tart and McNally, 1925.)

(3) *Static labyrinth reflexes acting on the extremities.* The compensatory nature of these reflexes is clear from Figs. 6 and 7. In the case of the fish it has been shown (Löwenstein, 1932) that the forced poses of the fins lead to an immediate righting of the animal as soon as any forward movement takes place. In the case of the frog the limb positions help in neutralising the effect of the slope of the substratum.

(4) *Tonic labyrinth reflexes acting on the trunk muscles.* In most cases, the labyrinthine effect on the trunk muscles becomes obvious only after unilateral labyrinth extirpation, and there is then an apparent asymmetry of the tonus of these muscles. It is difficult to decide whether this is the effect of a reflex deficiency or of a deficiency in the more diffuse labyrinthine tonic effect dealt with above (stimulatory tone).

All the static reflexes mentioned above can arise not only from a change in the animals' orientation to gravity but also, in the normal position, from fast rotation in the horizontal plane. In this case the resultant of the centrifugal force and the gravitational force acts as the stimulus which calls forth the static reflexes. Fast linear accelerations can have a similar effect (Tait and McNally, 1934).

(b) Dynamic reflexes.

Dynamic reflexes are compensatory effector movements which are called forth by angular accelerations during rotations and by linear accelerations during linear translations of moderate speed. They are due to twitch-like actions of the effector muscles and differ from the static reflexes, also by the shortness of their latent period.

(i) *Reactions to angular accelerations during rotations around the three main axes of the animal and around any resultant axis.*

(1) *Eye reactions.* (a) Horizontal eye movements: the eyes move in a plane of symmetry going through their anterior and posterior poles. (Response to rotations in a horizontal plane without change in orientation to gravity.)

(b) Rotatory eye movements: the eyes rotate around an axis going through the centre of the iris and the centre of the retina (Fig. 3).

(c) Vertical eye movements: the eyes rotate in a plane of symmetry going through their superior and inferior poles (Fig. 4). Movements (b) and (c) can lead to the tonic eye poses, described as rotatory and vertical eye deviations, if the movement of the animal is stopped when it is not normally orientated in relation to gravity. The effects of rotation and of change of orientation to gravity are superposed on one another in giving rise to the eye movements (b) and (c). Again, in animals with lateral eyes, movement (b) occurs during rotation around the transverse axis of the animal, movement (c) during rotation around its longitudinal axis; in animals with frontal eyes movement (c) replaces movement (b) and *vice versa*.

All the eye movements just described are directed *against* the direction of the rotation. They are compensatory in so far as they tend to maintain the visual field unchanged for at least a certain time during the rotation. If the rotation goes on, so that compensation becomes impossible because of the limited mobility of the eyes, the eyes jerk backward into their normal position and a new compensatory movement follows. This rhythmic alternation of compensatory movement and quick backward jerk is called nystagmus. The compensatory phase is always directed against the direction of the rotation, the quick phase in the same direction

as the rotation. The direction of a nystagmus is usually described according to the direction of the quick phase.

After cessation of fast passive rotation an after-nystagmus of the eyes may occur (post-rotatory nystagmus), the quick phase of which is now in the opposite direction to the preceding rotation. These after-reactions can be regarded as "unnatural" reactions, occurring only under experimental conditions, and they are caused only by rotations faster than those occurring during normal locomotion.

(2) *Head reactions.* In animals which can move the head, compensatory head movements occur during rotations around any of the axes mentioned above. They are directed against the direction of the rotation and, in their compensatory effect, collaborate with the eye reactions. Nystagmus and after-nystagmus of the head may also occur.

(3) *Reactions of the extremities.* The reactions of the extremities to rotations are also compensatory in effect. They tend to damp spontaneous rotations and to annihilate the effect of passive rotations. They are the least conspicuous of the dynamic reflexes.

(ii) *Reactions to linear accelerations.*

Reactions of the head, the limbs, and the eyes to linear accelerations are known in all vertebrates except fishes and reptiles.

(1) Head lifting and lowering have been described as lift reactions.

(2) In birds and mammals so-called landing reactions of the limbs take place during vertical downward movements.

(3) Certain eye reactions have been shown to take place in the guinea-pig during lateral linear translations.

The actual stimulus during linear translations is a linear acceleration. Gravity and centrifugal force can be regarded as linear accelerations too. It has been shown in the frog by McNally and Tait, that fast linear translations, like the centrifugal force, can give rise to reactions identical with the static gravity responses.

The labyrinthine origin of all the reactions enumerated above has been demonstrated for all vertebrate classes in numerous pieces of experimental work. These reactions are at least temporally abolished by bilateral labyrinth extirpation, provided that visual orientation be excluded. Even, then, some of the reflexes can be called forth by contact and proprioceptive stimuli.

IV. THE LOCALISATION OF THE EQUILIBRIUM FUNCTION WITHIN THE LABYRINTH.

Owing to the practical difficulties involved in eliminating separately the different sense endings within the labyrinth, the part played by each sense ending in relation to the known labyrinthine reflexes is still partly a matter of theory or conjecture.

The well-known theory of labyrinth function evolved by Mach (1875), Breuer (1889, 1891) and Brown (1873-4) has been disproved in an essential point by a number of experimental results. In his theory of the equilibrium function of the labyrinth Breuer started from the assumption that *all three* otoliths were equally

concerned in the production of the gravity responses and of the responses to linear acceleration. This assumption was adopted in all later theoretical work of importance (e.g. Magnus and de Kleijn, 1924, 1926*a*; Quix, 1924; de No, 1926, 1931). These authors tried, in accordance with Breuer's conception, to attribute certain reflexes to certain otoliths. The parallel in spatial arrangement between the semi-circular canals and the three maculae, as pointed out by Breuer, played an important, but unfortunately misleading, part in these analyses of labyrinth function. Results obtained from isolated mechanical stimulation of the different otoliths (Kubo, 1906) seemed to confirm Breuer's assumption.

As soon as operative elimination of single sense endings or groups of sense endings was successfully carried out, it was shown that the assumption that all the otoliths participate in giving rise to gravity responses is incorrect. There is now experimental evidence of this from all vertebrate classes.

(a) FISHES.

In selachians (e.g. *Mustelus canis*) the sacculus can be put out of action without damage to the remaining parts of the labyrinth. This operation is not followed by any disturbance of the known equilibrium reactions nor by any loss in muscle tonus (Parker, 1909; Maxwell, 1919, 1923). This is also true for the flat-fish *Pseudopleurinctes platessa* (Lyon, 1900).

Manning (1924) was able to destroy the pars inferior (sacculus and lagena) of the gold-fish, *Carassius auratus*, without interfering with the remaining parts of the labyrinth. He, too, did not find any disturbance of balance during spontaneous movements. His experiments were, however, done on seeing (not blinded) fishes only, and he did not analyse their equilibrium reflexes.

The same result was obtained by Parker (1908) on *Cynoscion regalis* and by Werner (1929) on *Gobius joso*, where a complete anatomical separation of the pars superior and the pars inferior facilitates operative elimination of either of the two parts. The significance of these experiments was, however, affected by the lack of control experiments with blinded animals and of a detailed analysis of their reflexes after the operation.

As in many vertebrates, in *Phoxinus laevis* the pars superior is connected with the pars inferior by only a very narrow membranous communication, the canalis utriculo-saccularis, an arrangement which again permits operative elimination of either part without damage to the other. Using an operative method worked out by v. Frisch (1929, 1931), I was able to establish that in *Phoxinus* none of the known labyrinthine reflexes is in any way affected by a bilateral extirpation of the pars inferior (sacculus and lagena) (Löwenstein, 1932). Bilateral extirpation of the pars superior (utricle and semicircular canals) is followed by a complete loss of all labyrinthine reflexes, loss in muscle tone and complete disorientation of movement, and thus is equivalent in its effect on equilibrium to a bilateral total labyrinth extirpation.¹ These experiments were done both on seeing and on blinded animals.

¹ After the extirpation of the pars superior, the pars inferior was undamaged and still functional, for there was no disturbance of the fishes' reactions to sound (v. Frisch and Stetter, 1932).

The animals were under observation for several months, and during that time their equilibrium reflexes were repeatedly examined. Moreover, all operations were finally checked by histological examination of serial sections.

It can be said, therefore, that in fishes the pars inferior of the labyrinth with its two maculae (sacculus and lagena), each bearing a large otolith, is not concerned in the production of any of the known labyrinthine reflexes.

V. Frisch and Stetter (1932) showed that in *Phoxinus* the pars inferior is the seat of the auditory function.

(b) AMPHIBIA.

Earlier investigations on the frog and the axolotl (Laudenbach, 1899; Maxwell, 1924; Huddleston, 1928) provide evidence in the same direction. In experiments on *Rana sylvatica* and *R. pallustris*, McNally and Tait (1925) were able to put the sacculus out of action by cutting the supplying nerve branch. Reflex tests on seeing and on blinded animals showed that in the frog this operation is without any effect on the equilibrium reflexes.

Interesting support for these findings has been established by Ashcroft and Hallpike (1934 *a, b*) by means of an entirely different method. They severed the sacculus nerve (frog) and took oscillograph records from its distal part. Rotations and tilting did not produce any excitation in this nerve, whereas the authors were able to get complete reproduction of sounds up to 512 vibrations per second. It follows that in the frog also the sacculus is not concerned with equilibrium reflexes, but acts as receptor for sound vibrations.

(c) REPTILES.

Tait (1932) states that "ablation of the saccules in a rattle-snake causes no detectable disturbance of equilibrium or of posture". No detailed account of this experiment, however, has been published.

(d) BIRDS.

The first separate elimination of an otolith organ in birds was done by Breuer and described in his classical work on the function of the otolith organs (1891). Breuer extirpated the cochlea plus lagena in the pigeon and found that this operation was not followed by any disturbance of equilibrium. Unfortunately, he did not pay any attention to these findings, and on the basis of comparative anatomical considerations postulated nevertheless a participation of this macula in the production of the responses to vertical linear accelerations.

Benjamins and Huizinga (1927, 1928 *a, b*) succeeded in the separate elimination of the pars superior and the pars inferior in the pigeon. They found that the loss of the sacculus and the lagena has almost no effect on the equilibrium reflexes and none at all on muscle tone. Bilateral extirpation of the utricle and the semicircular canals is almost equivalent to bilateral total labyrinth extirpation. Only a single reflex seems to be affected by the extirpation of the pars inferior and to remain

after the extirpation of the pars superior. It is the rotatory eye deviation occurring when the head is in an upward or downward position (p. 118). The work was supported by careful reflex tests in seeing and blindfolded animals and by quantitative measurements of muscle tone (pp. 135-6). The conclusion of the authors from these experiments is that in birds the pars inferior (sacculus and lagena) has, if any, only a very subordinate equilibrium function (also Benjamins, 1934).

(e) MAMMALS.

Great difficulties in the operative approach to the labyrinth of mammals seemed for a long time to render a separate otolith elimination impossible. At last Versteegh (1927) was successful in the separate elimination of the sacculus in the rabbit.

Elaborate reflex tests showed again that after this operation all known labyrinthine reflexes can be normally evoked. Versteegh, like Benjamins and Huizinga, describes, however, a slight effect on the rotatory eye deviation, but admits the possibility of a slight injury to the pars superior. The counter-check of a separate extirpation of the pars superior was unfortunately impossible.

Thus a series of experiments on fishes, amphibians, birds, and mammals have consistently shown that only one out of the three otolith organs in the vertebrate labyrinth, namely the utriculus, is concerned with labyrinthine reflexes and muscle tone. The otoliths of the sacculus and the lagena have very probably no equilibrium function at all.

Breuer's theory of otolith function, as far as it deals with the distribution of function over the labyrinth, has therefore been proved to be erroneous, and, with it, all the later theories based on Breuer's assumption.

It may be of interest in this connection to quote a passage written by de Kleijn (Magnus and de Kleijn, 1932) in a supplementary reviewing article in *Bethe's Handbuch*, in which he alludes to the experimental results just described: "Diese experimentellen Tatsachen bringen alle Theorien, welche sich zur Erklärung der tonischen Labyrinthreflexe auf die Zug- resp. Drucktätigkeit der Utriculus- und Sacculusmembranen stützen, zu Fall (Theorie von Magnus und mir, Theorie von Quix). Momentan fehlt sogar jede Theorie, welche die Auslösungsweise der verschiedenen Labyrinthreflexe auch nur einigermaßen in Einzelheiten erklären könnte."

The balance labyrinth is therefore a smaller unit than was previously supposed. It is identical with the pars superior and contains the semicircular canals and the utriculus.

V. THE FUNCTION OF SEPARATE PARTS OF THE PARS SUPERIOR.

(1) METHODS OF APPROACH.

Separate elimination of the different parts of the pars superior is rendered very difficult by the closeness of the sense endings and the wide communication between the membranous cavities in which they lie. Separate extirpation in which it is

necessary to open the membranous labyrinth can hardly be done without damaging adjacent parts. Experiments of this kind are therefore of only limited significance. Much more reliable are experiments in which the method of separate nerve cutting has successfully been applied in the study of the function of single semicircular canals. Only in one case, however, has it been possible to use it in eliminating the utriculus (Tait and McNally, 1934, on the frog).

A completely different method of elimination of otolith organs has been developed by Wittmaak (1909). By fast centrifuging of the guinea-pig he succeeded in throwing the relatively heavy otolith membranes off their maculae, while little or no damage was done to the cristae of the semicircular canals, which have a lower density. This method has been used in the study of the otolith function by de Kleijn and Magnus (1921), Hasegawa (1931) and de Kleijn and Versteegh (1932) in the guinea-pig.

Another way of approach is by the separate mechanical, thermal, or electrical stimulation of the different sense endings and the observation of the resulting reflexes. The disadvantage of this method, however, is well illustrated by the work of Kubo (1906) on the labyrinth of the dogfish. In this, mechanical stimulation of the sacculus also called forth distinct reactions which according to the facts described in the preceding section must have been due to a simultaneous stimulation of the utriculus or the semicircular canals. In electrical and to a certain extent also in thermal stimulation a direct effect of the stimulus on the main nerve tracts is apt to lead to erroneous results.

The experimental difficulties just described may explain the fact that, in spite of numerous experimental attempts, the function of the different sense endings within the pars superior is still far from being well understood.

(2) EXPERIMENTAL RESULTS.

Of the great number of earlier experiments on the function of different sense endings of the pars superior, which have already been repeatedly reviewed (Maxwell, 1923; Fischer, 1926; Magnus and de Kleijn, 1926*a*), only those will be dealt with here again which are based on sufficiently reliable experimental methods. Any method should include at least some of the following points, if the results are to be significant: a good operative technique, post-mortem or histological check of the effect of the operation, thorough reflex examination over a sufficiently long period (to exclude the immediate irritative effect of the operation), controls with blinded or blindfolded animals, and a clear distinction between dynamic and static reflexes in the reflex tests.

One of the most important questions at the moment is, whether there is in fact a significant difference between the function of a crista ampullaris and a macula. Is one, in fact, entitled to say that the semicircular canals are dynamic and the otolith organs static receptors only?

(a) *Fishes.*

In selachians bilateral separate elimination of the otolith organs without apparent damage to the ampullae of the semicircular canals (no reliable post-mortem controls were done) is followed by a loss of the gravity responses (tonic eye and fin poses) and loss of the general orientation as to the direction of gravity, as shown by a loss of the strong righting reflexes present in normal animals (Loeb, 1891; Kreidl, 1892, 1893; Lee, 1894, 1895). Experiments of the same kind were described by Maxwell (1919, 1920 *a, b*, 1923). He opened the labyrinth cavity and removed the otoconia from the otolith membranes by washing them off carefully with a jet of water, any damage to the ampullae being carefully avoided. In contradiction to the results mentioned above, Maxwell claimed that besides complete preservation of the dynamic reflexes and very little disturbance in balance during spontaneous movement, compensatory eye *poses* (static reflexes) could still be elicited after this operation.

There are, however, two facts which may perhaps explain this discrepancy in results. Maxwell laid very much emphasis upon the precautions he took to avoid damaging the ampullae and to disturb as little as possible the structure of the maculae of the otolith organs. It will be seen later that it has been claimed recently that in mammals (guinea-pig) a macula from which the otolith membrane has been removed can still give rise to tonic effector poses (p. 138).

Maxwell, however, drew the conclusion that the semicircular canals can give rise to static reflexes too.

In the complementary experiment, where Maxwell extirpated the ampullae of the semicircular canals only, he found that, in addition to retaining the static reflexes, the animals still responded to rotations around the transverse and longitudinal body axis with eye *movements* which were, however, much slower than those known from normal animals. Thus the only difference between a dogfish without otoliths and one without semicircular canals was that in the latter the reactions to rotations around the vertical dorso-ventral axis were missing.

From that Maxwell concluded that the otolith organs can also give rise to dynamic reflexes except during and after rotation around a vertical axis.

Maxwell therefore claimed that there is no fundamental difference in function between the otolith organs and the semicircular canals (at least the vertical ones). Each of these structures is both a gravity and rotation receptor.

Unfortunately Maxwell seems not to have distinguished carefully enough between the swift compensatory eye movements and those slow movements which are in any case necessary to lead the eyes up to any compensatory eye pose. Maxwell actually saw that the eye movements in question were slower than in an animal with intact semicircular canals, but he did not pay any attention to this difference.

Experiments where the otoliths of one side only were extirpated (Loeb (1891) in *Scyllium canicula*; Lee (1894) in *Mustelus canis*) showed that unilateral loss of the otolith organ is followed by forced poses of the eyes and fins, and by an asymmetry of the tonus of the trunk muscles. Similar results were described by

Benjamins (1920) in the carp and perch. The tonus asymmetries, mainly of the trunk muscles, provide evidence that the otolith organs (utricle) at least participate in the regulation of general muscle tone. Reflex tests have shown that there is also a decrease in the static eye reflexes when the animals are turned towards the operated side (Loeb, 1891; Lee, 1894; Benjamins, 1920). This shows the participation of the otolith organs in the static function. The fact that there is only a decrease and not a complete loss of the static reflexes when the animal is tilted towards the operated side shows that the remaining labyrinth is alone sufficient for the production of static gravity responses to inclinations around the longitudinal axis towards *both* sides. This has also been shown by Löwenstein (1932) in *Phoxinus*. Immediately after unilateral operation there appears to be a complete unilateral loss of static reflexes. This is probably due to the strong asymmetry in general muscle tone, interfering with the already weakened static reflexes to inclinations towards the operated side. The general tonus asymmetry becomes more and more neutralised, presumably by central compensation (Bechterew, 1883; Spiegel and Démetriades, 1925), and then the above-mentioned ability of the labyrinth (utricle) to act both ways becomes apparent. Thorough analysis of this process of "recovery" in a suitable animal might lead to the disentanglement of the complicated question of the labyrinthine influence upon both general muscle tone and static reflexes.

The method of mechanical stimulation of the utricle was used by Maxwell (1920 *a, b*, 1923). This author put the ampullae and the pars inferior out of action, so that the utricle alone was left intact. By pushing the utricle otolith in different directions, Maxwell obtained eye reactions corresponding to those shown in a normal fish when it is turned around its transverse and longitudinal axes (Figs. 3 and 4). For instance, moving the utricle otolith towards the left or right caused vertical eye deviations; moving the otolith forward or backward caused rotatory deviations of the eyes. These deviations continued as long as the otolith was held in its altered position.

Maxwell's conclusions from these experiments in regard to the mechanism of otolith function cannot be dealt with here. It should be noted, however, that it was possible to obtain eye deviations of both kinds, rotatory and vertical, from stimulation of the utricle only. This finding first supports the results dealt with in the preceding section in which it was shown that the utricle is the only otolith organ which gives rise to labyrinthine reflexes. It shows further that the utricle can rightly be regarded as responsible for static reflexes. Maxwell, however, comes again to the assumption of an additional dynamic function of the utricle, referring to the fact that he has seen eye movements taking place when the otolith was being moved. It must be pointed out again that it is impossible for an effector to assume a static pose different from its natural one without moving at all. The criteria for real dynamic reactions were not applied by Maxwell to the eye movements observed.

No further work has been done on the function of the isolated utricle in fishes, so that the question of whether it is simply static, or simply dynamic or both

static and dynamic in function, is not yet settled. The utricle does participate in the production of the static reflexes and in the maintenance and regulation of muscle tone. Whether it is the only part of the pars superior involved in these functions can be decided only by separate elimination of the semicircular canals.

Such experiments have been done by Lee (1894), Bethe (1894), Lyon (1899, 1900) and Maxwell (1910, 1919). Lee's experiments were done by the method of nerve severance which appears to be the most reliable one.

Bilateral elimination of the horizontal canals is followed by side-to-side pendulations during straightforward swimming, and these pendulations can lead to circus movements towards either side. After bilateral elimination of the anterior vertical canals the fish sometimes stand vertically head downwards, the eyes then showing a lasting rotatory deviation with their anterior poles pointing ventralwards. This is a forced eye pose which occurs in a head upward position in the normal animal. The reverse happens after bilateral elimination of the posterior vertical canals. After this operation back somersaults may occur. After unilateral elimination of the anterior and posterior vertical canals of the same side the fish in swimming show a list towards the operated side accompanied by vertical eye deviations similar to those exhibited in normal fishes when tilted towards the opposite side. Diagonal canal elimination (elimination of the anterior vertical canal of one side and the posterior vertical canal of the other side) results in a swaying movement, the pendulations taking place in the common plane of the eliminated canals.

The results obtained from canal elimination in fishes suggest that the semicircular canals in the normal animal give rise to compensatory effector movements, checking spontaneous or passive rotations taking place mainly in their own plane. The horizontal canals control circular movements in a horizontal plane, the anterior vertical canals check head downwards, the posterior ones head upwards movements around the transverse axis of the body. Sideways tilting around the longitudinal body axis appears to be checked by the action of the anterior and posterior vertical canals of each side working together. In the check of diagonal swaying one anterior and the crossed posterior vertical canal seem to collaborate. The forced eye poses occurring after the elimination of vertical canals, as described by Lee, suggest a tonic influence of the canals on the eye muscles.

Further investigations of the canal function based on thorough reflex analyses are necessary before anything more definite can be said as to their possible influence on tone and on static reflexes.

(b) Amphibia.

A thorough analysis of the function of separate sense endings of the labyrinth of the frog has been carried out by Tait and McNally. The work of these authors is especially valuable because of the reliability of the methods applied. It has been pointed out above that McNally and Tait proved that in the frog only the pars superior of the labyrinth is concerned with equilibrium reflexes and muscle tone (1925).

Using the method of nerve severance these authors succeeded in putting the sense endings of the pars superior out of action in any desired combination. Histological checks showed that this was done without inflicting any damage on adjacent parts. For the first time they also succeeded in a separate elimination of the utricle by nerve severing (McNally and Tait, 1933; Tait and McNally, 1934).

It would lead too far to describe all the experiments done. It is, however, necessary to deal in detail with a few fundamental results which throw new light upon the problems dealt with in this review.

Method of examination of the animals before and after operation.

(1) Observation of spontaneous behaviour.

- (a) On land.
- (b) In shallow and in deep water.

(2) Reflex tests.

- (a) Righting reaction from prone to supine.
- (b) Reactions to slow tilt on the tilting table around horizontal axes, that is to say, around the transverse, longitudinal and diagonal axes. (Static reflexes of the head, limbs and body.¹)
- (c) Two grades of quick tilting around the same axes (dynamic reflexes).
- (d) Reactions to passive head lift and drop. Chin lift and drop test. (Dynamic reflexes.)
- (e) Slow and very fast rotation around the vertical dorso-ventral axis on the turntable. (Dynamic reflexes and centrifugal reflexes.)
- (f) Rapid linear translations. (Reflexes to linear acceleration.)

The reflexes thus studied have been divided by the authors into two groups:

(1) Reflexes to slow changes in position in relation to gravity, and reflexes to centrifugal and linear acceleration. The latter two forces are in effect equal to a change in direction of the gravitational force (resultant law). (Static reflexes.)

(2) Reflexes to different grades of angular accelerations too slow however to produce a centrifugal effect. (Dynamic reflexes.)

Two types of operative eliminations within the pars superior are of particular interest in regard to the question of the seat of origin of static and dynamic reflexes:

(1) Elimination of all six semicircular canals, the only sense endings remaining in function being those of the two utriculi. (Bi-soli-utricular animals.)

(2) Separate elimination of the utricle on one or both sides. (Uni-de-utricular and bi-de-utricular animals.)

Elimination of single semicircular canals, sometimes in combination with the utricle of the same or of the opposite side, has also been done and will be dealt with, where necessary, in connection with the others.

¹ No eye reflexes have been studied throughout, the animals being blinded by extirpation of the eyes.

Bi-soli-utricular animals.

The main symptoms in these animals, which are due to the fact that the function of the utricle appears uninterfered with by canal function, are the following:

(1) The static reflexes are undamaged. From that alone, however, it cannot yet be decided whether the utriculi are the only part of the pars superior concerned with these reactions.

(2) The reactions to slow rotations around the vertical dorso-ventral axis are missing. This is attributed to the elimination of the horizontal canals.

(3) Rapid tilting around the horizontal axes gives rise to vehement head and body movements *in* the direction of the passive movement. Instead of being compensatory these reactions lead to upsetting the animal forwards, backwards or sideways. From the fact that these reactions are missing in completely delabyrinthised animals, the authors concluded that they arise from the utriculi. This would mean that the utriculi in fact respond to fast angular accelerations (dynamic response). This response is, however, reversed in direction to the response of the semicircular canals under the same circumstances. Tait and McNally assume that in the normal animal these reactions have a damping effect on the compensatory reflexes arising under these conditions from the vertical semicircular canals. The results obtained in combined eliminations of single vertical canals and the ipsi- or contralateral utricle have shown that the utricle reacts antagonistically to the contralateral vertical canals.

(4) During spontaneous forward movements the bi-soli-utricular animals exhibit a tendency to vehement pendulation around all possible horizontal axes. These pendulations can also be studied by means of the chin lift and drop test. The explanation given by the authors for these pendulations is that they cannot be considered to be a pure deficiency phenomenon due to the mere absence of the compensatory effect of the eliminated canals. On the contrary they must be considered as a positive effect called forth by the utriculi, which only appears when the antagonistic vertical canals are put out of action. This explanation can perhaps be applied to the pendulations described in other vertebrates (*e.g.* birds, see p. 137) as pure deficiency phenomena due to the elimination of vertical canals. The stimuli calling forth these pendulations can be considered to be those passive angular accelerations of the head which normally are bound to occur during any spontaneous body movement. In the normal animal they are doubly checked, first by the compensatory reactions called forth by the vertical canals, while these themselves are being counter-checked by this newly described action of the utriculi.

Animals in which one utricle and the six semicircular canals have been eliminated (uni-soli-utricular animals) provide proof for a tonic influence of the utricle. These animals show an asymmetrical forced pose similar to that shown in animals after unilateral total labyrinth extirpation. These tonic disturbances also affect the trunk muscles. The righting reaction is deficient. When, however, the animal succeeds in righting itself from supine to prone, it does so by rolling invariably with the side of the intact utricle lowermost. Reflex tests have shown,

however, that lateral tilt in either direction still calls forth static reflexes, tilting towards the side with the intact utriculus calling forth a stronger reaction than that towards the side without utriculus. This is in accordance with the results, which I have described (Löwenstein, 1932) in the minnow. It shows that one utriculus can react both ways, the stronger effect being produced, however, in a changed position (around the longitudinal axis) by the lowermost utriculus (compare p. 128).

Bi-de-utriculate animals.

The results obtained with animals of this kind are of great importance because no such operation has previously been successful in any vertebrate.

(1) All static reactions are lost. This again furnishes proof for the static function of the utriculi and against a static function of the semicircular canals (compare pp. 127 and 129).

(2) Tilting around any horizontal axis is instantaneously compensated by counter-movements of the head and limbs (dynamic reflexes), the effect of which is that the head during these rotations maintains an approximately horizontal orientation in space. The loss of the utriculi, the damping effect of which on the compensatory effector movements, called forth by the vertical canals, has been discussed above, really manifests itself now by a tendency to over-compensation of angular accelerations which are well above the threshold of the canals. An interesting phenomenon in this connection is a striking tremor of the head during, and more particularly after, fast spontaneous movements. It consists of jerky up and down pendulations of the head. As shown by subsequent elimination of vertical canals, this tremor is due to an alternating stimulation of opposite vertical canals, called forth by passive head rotations during the spontaneous movement. It shows clearly the loss of utriculus damping on the rapid alternation of successive compensatory movements elicited in consequence of successive stimulation of antagonistic canals.

(3) Bi-de-utriculate animals show a considerable tendency to thigmotactic reaction, which shows itself as a trapping effect of obstacles encountered by the animal during locomotion in water. This may be ascribed to the absence of gravity orientation.

(4) There is, further, an interesting tendency of the animals temporarily to "fall asleep" in deep water. This state of "sleeping" is accompanied by a decrease of frequency of the respiratory movements. Tait and McNally abstain from an explanation of the phenomenon. It seems, however, quite reasonable to attribute it to a loss in general labyrinthine stimulation (p. 117). This would be evidence for the participation of the utriculus in the stimulatory function of the labyrinth.

(5) Rotation around the vertical dorso-ventral axis (turntable) is still followed by clear and undiminished compensatory head movements and after-reactions in a horizontal plane, accompanied by body curve (horizontal canals). During and after fast rotations, lateral inclination of the head and body occurs too. This

reaction, called the spinal torque, is attributed by the authors to a stimulation of the posterior vertical canals by high angular accelerations in a horizontal plane. The results of uni- and bilateral additional elimination of the posterior vertical canal confirmed this assumption.

Uni-de-utriculate animals.

Again in these animals there is an asymmetry in tone giving rise to a forced pose similar to that known from unilaterally delabyrinthised or from uni-soli-utricular animals. From the fact that in both the uni-soli-utricular and the uni-de-utriculate animals the tonus asymmetry is less than after total unilateral labyrinth extirpation, one may conclude that both the semicircular canals and the utriculi are responsible for the regulation of general muscle tone. This view is supported by the fact that in an uni-de-utriculate animal the tonus asymmetry is increased after an additional elimination of the vertical canals on the side of the severed utriculus nerve. Moreover, the tonus asymmetry can be decreased by an additional elimination of the vertical canals on the side of the undamaged utriculus. There seems therefore to be an antagonism between the utriculus and the contralateral vertical canals in regard to tonus as well as in regard to reflex reaction.

Only the most important details of Tait's and McNally's results could be dealt with here. The general conclusions from them may be summarised as follows:

(1) In the frog the utriculi are the only seat of the responses to gravity, centrifugal force and linear acceleration (static reflexes).

(2) The vertical semicircular canals respond to angular accelerations around any horizontal axis by giving rise to compensatory effector movements (dynamic reflexes). Single vertical canals are stimulated mainly by rotations in their own plane. There is, however, certain evidence for their stimulation even by rotations in a horizontal plane.

(3) The great efficiency of the semicircular canals during fast rotations would lead to over-compensation, were it not prevented in the normal animal by a damping counter-reaction called forth by the utriculi under the influence of angular accelerations. Owing to this function the utriculi must be described as having a dynamic function too, but not in the sense that Maxwell postulates (1923). The utriculi do not collaborate with the vertical canals, they are their antagonists. They cannot therefore replace canal function in its compensatory effect.

(4) There is no evidence that the vertical canals are able to give rise to any static reflex pose; they seem, however, to take part in the regulation of general muscle tone.

(5) The horizontal canals are exclusively concerned with purely dynamic responses.

(c) Reptiles.

No work has been done concerning the function of single sense endings within the pars superior of the reptilian labyrinth.

(d) *Birds.*

A great number of elimination experiments have been carried out on the semicircular canals of birds (Fischer, 1926). In these experiments the canals were either cut or the ampullae were extirpated. As early as 1889 Breuer, in discussing such experiments carried out by Baginsky, wrote: "Ich glaube, dass nachgrade genug Bogengänge durchschnitten worden sind. Den 105 Tauben, welche Baginsky diesen Versuchen geopfert, könnten noch Hekatomben nachfolgen ohne dass unser Wissen dadurch vermehrt würde. Diese Versuche sind ja von Flourens schon vollständig erledigt worden. Das Sinnesorgan, welches das Vestibulum ja doch jedenfalls enthält, muss schliesslich mit feineren Methoden untersucht werden, als es das immer wiederkehrende Durchschneiden, Ausbrechen oder Verbrennen der Kanäle ist."

Even up to the present day it is not possible to use better methods, and in the recent work of Groebels (1926) and Huizinga (1931, 1932), carried out on hundreds of pigeons, the same method is still adopted in principle. No canal elimination by separate nerve severance has been carried out yet on birds.

Thus only a few consistent results are available. For instance, Groebels (1926) obtained different results according to whether he eliminated the canals by mere section of the canal or by extirpation of the ampulla. Huizinga (1932), by canal cutting only, again comes to conclusions different from those of Groebels in important points.

On the one hand it is quite possible that the mere cutting of a canal does not abolish completely the function of its ampulla; on the other hand, extirpation of an ampulla may affect the adjacent parts of the pars superior too. In both cases the outflow of endolymph may alter the whole functional capacity of the labyrinth.

In what follows it is mainly the results obtained by Huizinga that will be taken into account.

After opening the bony canals above the ampulla, Huizinga cut the semicircular canals of the pigeon at the ampullary end of the canal. He carefully avoided any traction on the canal and the other parts of the pars superior. Bilateral section of the horizontal canal was followed by horizontal head pendulation, and a slight rotation of the head around the oro-occipital axis of the skull. In forward progression the pigeons showed alternating circling to either side. On the turntable the horizontal after-nystagmus of the head was missing (the nystagmus during rotation was not recorded); there occurred, however, up and down movements of the head in alternation with slight sideways inclination to either side. These movements suggest that the intact vertical canals can be stimulated by turntable rotations too (cf. p. 133). Bilateral section of the anterior vertical canals was not followed by any head pendulations. There was, however, a tendency to somersaults forwards. No pendulations, but somersaults backwards, occurred after bilateral section of the posterior vertical canals. The horizontal after-nystagmus was in both cases normal, perhaps slightly affected in its duration.

The effect of the section of a pair of crossed vertical canals (the anterior canal on one side and the opposite posterior canal) was quite different. At once there were conspicuous head pendulations in the plane of the sectioned canals. The horizontal after-nystagmus of the head was again normal, except for a slight decrease in its duration.

Huizinga concluded from these results that the six semicircular canals form a functional system of three pairs of co-ordinated canals, the two horizontal canals and the two pairs of crossed vertical canals.

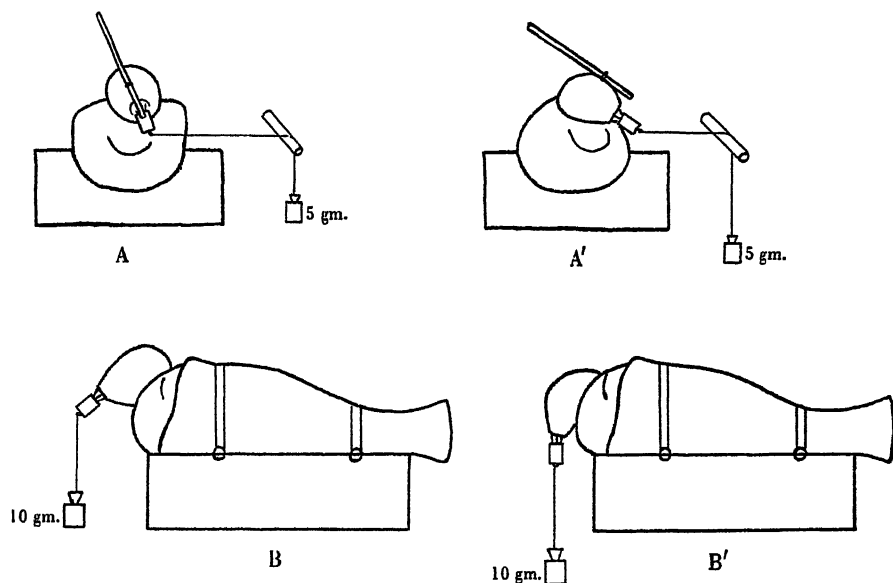


Fig. 8. Comparison of the resistance of the neck muscles of the pigeon to tensile stresses before and after section of semicircular canals. A and A' = resistance to sideways bending of the neck before and after section of the horizontal canals. B and B' = resistance to head dropping before and after section of a pair of crossed vertical canals. (Modified after Huizinga (1932).)

Like Ewald and Bechterew, Huizinga considers the head pendulations occurring after canal elimination as pure deficiency phenomena due to a loss in tone in the neck muscles.

Groebbels, on the contrary, describes them as the effect of a "primary irritation" arising from the operated ampulla or its nerve.

The lack of tone in the neck muscles was shown by Huizinga in experiments on the resistance of the neck to the strain of a weight in different directions before and after section of correlated pairs of canals. The method used is shown in Figs. 8 and 9.

Bilateral section of the horizontal canals was followed by a decrease in resistance to sideways bending of the head (Fig. 8 A, A'). Section of a pair of crossed vertical canals resulted in a decrease in resistance to passive head dropping and lifting (Fig. 8 B, B') or to rotation around the oro-occipital axis of the skull (Fig. 9).

Bilateral section of the same kind of vertical canals and section of any single canal did not significantly diminish the resistance of the neck muscles to the strain of a weight in any direction.

There was no noticeable decrease in the tone of the wing muscles after any of the canal eliminations. This was shown by Benjamins and Huizinga (1928 *a*) to occur after bilateral extirpation of the whole pars superior. The tone of the wing muscles may therefore be considered to be elicited by the utriculi. Groebbels, however, did observe a decrease in wing muscle tone, but only after *extirpation of the ampullae* (not after mere canal cutting). Whether this was due to the de-

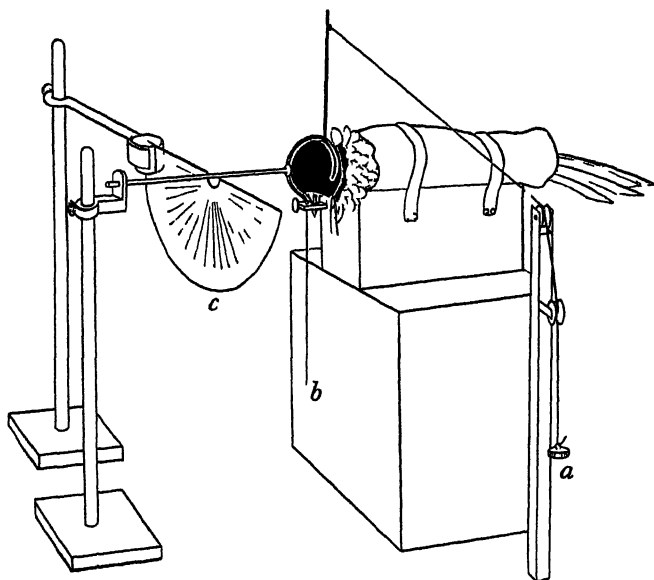


Fig. 9 Measurement of the resistance of the neck muscles of the pigeon to passive head rotation around the oro-occipital axis of the skull. (From Benjamins and Huizinga (1928 *a*)) The head is twisted by the weight *a*, and the movement of the pointer *b* is measured by the protractor *c*

struction of the ampullary sense endings or to an additional damage to the utriculi cannot be decided. Besides that, Groebbels found that certain *static* reflexes of the wings and the tail were affected by extirpation of the ampullae of the posterior vertical canals. These effects he explains as real deficiency phenomena due to the degeneration of the ampullary nerves. He therefore assumes not only a mere tonus regulating, but also a static function of the semicircular canals.

A further effect of canal elimination was described by Groebbels. After unilateral extirpation of at least two ampullae his pigeons showed the neck torsion first described by Ewald as occurring after total labyrinth extirpation (Fig. 10). Here Groebbels assumes a so-called "secondary irritation phenomenon", which in contrast to the primary ones (pendulations, etc.) only occurs after degeneration of the ampullary nerves. It is supposed to be due partly to stimuli from the intact ampullae, which become effective only after the cessation of inhibitions arising from the extirpated parts. Groebbels admits, however, that the latter phenomenon

also occurs in the absence of any ampulla, and he thinks, therefore, that other parts of the labyrinth (utricle?) may be involved too. On the whole Groebbels' results and their explanations are not very convincing, especially in view of the absence of any description of histological checks on the operations.

Returning to the results of Huizinga one can summarise them as follows:

(1) Canal section is equivalent to ampullary extirpation in its effect but prevents additional damage to other parts of the *pars superior*.

(2) All effects of canal elimination are pure deficiency phenomena due to the lack of function of the eliminated ampullae and not to any irritation from their nerves or their cristae.

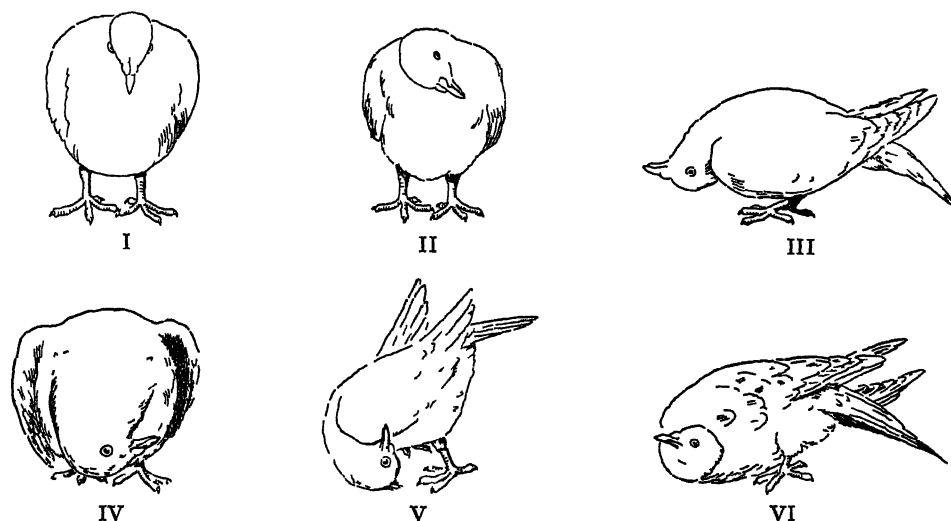


Fig. 10. Six stages of forced neck torsion in the pigeon after total extirpation of the right labyrinth. (After Ewald, from Fischer (1926))

(3) The pendulations after elimination of correlated pairs of canals are due to a "tonus" deficiency of the neck muscles. A tonic function of the semicircular canals must therefore be assumed.

In regard to the head pendulations and to the noted tendency to somersaults one might in accordance with the results of Tait and McNally (p. 131) postulate active interference by the utricle. This can only be decided, however, by separate elimination of the utricle: an operation that has not yet been carried out in birds. Very little attention has been paid in the work on birds to the labyrinthine reflexes. Following Ewald's theory of the "Tonuslabyrinth" most of the results obtained in birds are expressed in terms of muscle tone. The question of the nature of this "tone" will be discussed in the last section (p. 140).

(e) *Mammals.*

The results obtained from the human ear are deliberately omitted here, although some valuable evidence might be derived from them (Grahe, 1926). The experimental attack on the question of the localisation of the different functions within

the labyrinth is, however, completely limited to the study of reflex deficiencies in pathological cases, the cause of which can be only roughly localised during surgical operations and in occasional post-mortems.

But in other mammals too the experimental approach is very difficult. As stated above, separate elimination of the utriculus has only been carried out so far by rapid centrifuging in guinea-pigs (Wittmaak, 1909; de Kleijn and Magnus, 1921; Hasegawa, 1931; and de Kleijn and Versteegh, 1932). These experiments on the guinea-pig show that in mammals too the static reflexes are utriculus reflexes, whereas the reactions to angular accelerations arise from the semicircular canals. As to the seat of the reactions to linear accelerations, there is no agreement between the results of the different authors. De Kleijn and Magnus found these reflexes still present after the otolith membranes had been centrifuged off their maculae. They claimed, therefore, that they arise mainly from the semicircular canals. They agree, however, on theoretical grounds, that it is possible that the otoliths share this function with the canals. Hasegawa finds, on the contrary, that the reactions to linear accelerations are abolished when the otolith membranes are severed from the maculae by centrifuging.

Measurements of muscle tone after centrifuging have not been made. The following experiment, however, gives indirect evidence that the labyrinth deprived of its otolith membranes still exercises a tonic influence (Magnus and de Kleijn, 1922). In animals with severed otolith membranes, which showed no tonus asymmetry, one labyrinth was transitorily put out of action by an injection of cocaine. At once all the tonus asymmetries known from unilateral labyrinth extirpation appeared. The forced poses due to these tonus asymmetries could not be interfered with, however, by alteration of the position of the head in relation to gravity, as is the case after unilateral labyrinth extirpation. The authors assumed that the regulation of the general muscle tone is a function of the utriculus macula itself, whereas the tonic reflexes are due to a mechanism in which the weight of the otolith membranes acts upon the maculae. This would indeed be a very good explanation of the superposition of the two distinct functions of the labyrinth; but it has been shown recently by de Kleijn and Versteegh (1932) that tonic *reflexes* can still be called forth in the absence of the otolith membranes.

From that it would follow that the otolith membranes were not necessary even for the production of tonic reflexes.

Two arguments suggest themselves against these conclusions:

- (1) The general tonic effect ascribed to the maculae lacking otoliths may just as well be due to a tonic function of the semicircular canals.
- (2) It is still possible that in mammals the semicircular canals are capable of giving rise to tonic reflexes too.

Making use of Ewald's method of canal plugging, de No (1926, 1927) found that in the rabbit plugging of all six semicircular canals, besides causing a complete loss of the dynamic eye responses to rotations, also abolished, or at least modified, the static eye reflexes. Moreover, the eye reactions to linear acceleration during linear translations were abolished. After plugging of the horizontal canals the eye

responses to the action of centrifugal force were considerably modified. On the basis of these experiments, and on theoretical grounds, de No strongly favours the view that the semicircular canals take part in the production of tonic reflexes.

Canal elimination by nerve cutting has not yet been successfully performed in mammals.

(3) GENERAL CONCLUSIONS.

Conclusions as to the function of the otolith organs and the semicircular canals, valid for all vertebrate classes, are difficult to draw, because generalisations from results gained in any one vertebrate class are only acceptable for another with great caution. The differences in the morphology of the labyrinth as well as in the structure of the effector systems responding to labyrinthine regulations in the different vertebrate classes may perhaps render many of our generalisations futile.

Nevertheless, some general conclusions can safely be drawn from the experimental data described in the preceding sections. At the same time, some additional facts concerning the function of the semicircular canals will be discussed.

(a) *Otolith organs.*

The otolith-bearing macula utriculi is undoubtedly concerned in the maintenance and regulation of general muscle tone. The only question is whether it plays such a dominant part in tonus maintenance in the resting animal as has been attributed to it by the Utrecht school of workers. Tait and McNally are inclined to conclude that this is not the case (1934). They rather think that the maintenance of even the general tone is due to a mechanism of a more floating nature, that is to say, to a pattern of reflex reactions called forth by a continuous change in the state of stimulation of the different sense endings of the pars superior. They do so on the basis of the assumption that the head of an animal is never completely at rest, so that there is continuous stimulation of at least the canals. Besides that, visual and proprioceptive stimuli as well are involved in the maintenance of "reflex tone".

Production of the static reflexes and of the reflexes to centrifugal force and fast linear acceleration is in all probability the main function of the otolith organs (utriculi).

It has been shown that the utricle of the frog can be stimulated by fast angular acceleration. The same may be true for all vertebrate classes. Since the responses are quick effector movements, a dynamic function also must be attributed to the otolith organ.

The view that the otolith organ is, on the whole, a rather inert receptor (Magruss and de Kleijn, 1932; de No, 1927, 1931) is justified by two facts: first, that, during rotations of moderate speed, it cannot immediately check angular accelerations, as the semicircular canals do; and second, that *only* lasting or very strong stimuli call forth otolith responses.

(b) Semicircular canals.

In discussing Ewald's theory of the "Tonuslabyrinth", McNally and Tait (1933) quite rightly point out that the deficiencies in tone after labyrinth eliminations described by Ewald (and other workers) are not of a uniform kind, but fall into two different categories. It was shown, on the one hand, that in bilaterally labyrinthectomised pigeons certain muscle groups (*e.g.* the neck muscles) can resist only weakly the lasting strain of a weight. On the other hand, Ewald ascribed certain deficiencies in tone to a loss in the capacity for quick action ("Verlust der Möglichkeit schnell zu handeln"). If, after bilateral labyrinth or canal elimination, a pigeon is shaken by hand, the head follows all the shaking movements without resistance. That means that the animals are unable to counteract these passive head movements instantaneously by compensatory dynamic reflexes. This second kind of "tonus" deficiency also described by Groebbs (1926) and Huizinga (1932) may, of course, be nothing but the loss of dynamic responses arising in the normal animal from the semicircular canals.

The semicircular canals are quick receptors, that is to say, they react with little inertia, and the reactions set up by them have a very short latent period (de No, 1927, 1931; Magnus and de Kleijn, 1932).

Steinhausen (1931, 1933*a, b*) was able to observe the movements of the cupula in the ampullae of the labyrinth of the pike during passive rotations and during thermal stimulation, and he states that the cupula reacts instantaneously to the slightest stimulus; that is to say, it shows very little inertia in the uptake of a stimulus.

Tait and McNally (1929) studied quantitatively the latent period of the frog's gastrocnemius reaction to passive angular accelerations. This reaction is set up by the semicircular canals (as shown by its disappearance after canal elimination). When a frog is suddenly tilted backwards on the tilting table, its gastrocnemius at once contracts. The effect of this contraction in the normal animal would be an instantaneous damping of the backward movement of the body. The latent period was a minimum of 35 msec. (magnitude of known tendon reflexes). Buchanan (1908) has shown that the time taken by a nerve impulse to pass a single synapse in the frog is between 12 and 21 msec. Tait and McNally therefore concluded that only two synapses are involved in the nervous path of these canal reflexes. The capacity for quick reaction due to the semicircular canals has therefore its physiological basis in the shortness of the reduced reflex time of the canal reflexes.

As it was shown in the preceding sections during active movements of the animals, the vertical canals come into play mainly when the position of the head in relation to gravity changes. Their quick uptake of any curved head movement around a horizontal axis makes them act as "heralds". The compensatory movements of the effectors caused in this way either immediately damp the head or body movement, or, in the case of passive head or body displacement, lead to forced compensatory poses of the effectors. After the cessation of the movement

in an unnatural head or body position, the forced poses are tonically upheld by static reflexes originating mainly in the otolith organs (utricle).

Thus a functional connection exists between vertical canals and utricle, which, as shown above (p. 133), can also extend to a direct dynamic interaction of the two structures. A real static function of the vertical canals has been claimed on experimental grounds by Maxwell (1919, 1920*b*) and de No (1926) on the basis of experiments on fishes and mammals.

The purely dynamic function of the horizontal canals has never been doubted by any worker.

Two questions remain to be discussed in connection with canal function:

(1) It has been shown that the horizontal canals give rise to reactions to rotations around the vertical dorso-ventral axis. Among these reactions eye reflexes play a considerable part. The so-called horizontal eye nystagmus which is a purely dynamic reflex is considered to be set up mainly by the horizontal canals. The question now is: do the vertical canals also give rise to nystagmic eye reactions? If so, under what circumstances and in which plane do they occur? Unfortunately Tait and McNally in their thorough study of the function of the vertical canals in the frog have not dealt with any eye reactions whatsoever. Their animals were blinded by extirpation of the eyeballs.

The only experimental evidence in connection with this question therefore depends upon experiments with isolated mechanical or thermal stimulation of different ampullae. It has been shown in fishes, birds and mammals (cf. Fischer, 1926; Magnus and de Kleijn, 1926*a*; de No, 1931) that by stimulation of vertical canals rotatory and vertical eye movements and nystagmus can be called forth. Stimulation of the horizontal canals always results in horizontal eye movements or nystagmus. The disadvantages of the stimulation method which have been discussed above (p. 126) demand a revision of these results by experiments with separate nerve severance.

During reflex tests when the animals are rotated around any horizontal axis, the compensatory eye movements are always superposed upon the eye deviations due to the change of the animal's orientation in relation to gravity (static reflexes). In yet unpublished experiments on the purely dynamic eye reflexes of fishes (tench and pike) I have found that, during rotation on the turntable, a fish, which is fixed in any possible position in space, shows only one kind of eye nystagmus and after-nystagmus. The eyes always oscillate nystagmically in a plane of symmetry of the eyes which goes through their anterior and posterior poles, whatever forced pose the eye may exhibit due to the particular orientation of the animals in space.

As to the plane in which a given semicircular canal reacts, it is safe to say that the maximum reaction is produced by rotations in its own plane. Reactions to rotations in other planes even around an axis which represents a chord of the arch of the canal seem possible. The stimulation of the vertical canals, by rotations around the vertical dorso-ventral axis (frog, Tait and McNally, see p. 133) seems to provide sufficient evidence for that.

(2) The last question to be dealt with here in connection with canal function

is a matter of an old controversy. Can a given semicircular canal, during rotation in its own plane or nearly in it, respond to rotations in both directions, that is to say, when the ampulla is going ahead and when it is following the canal, or to rotation in one direction only?

Breuer and Ewald were definitely in favour of a both-ways response of the canals, but described one direction of rotation as dominating in effect over the opposite one. In the horizontal canals the stronger stimulus was supposed to be supplied, when the ampulla is following the canal. The reverse was said to be the case for the vertical canals.

In fishes, birds and mammals, experimental evidence has been provided for this view of Breuer and Ewald from unilateral total labyrinth extirpations. Even in blind representatives of these three vertebrate classes, head and eye reactions and after-reactions can still be called forth by rotations in either direction about the vertical dorso-ventral axis. The effect of rotations towards the side of the intact labyrinth is much greater than the effect of the opposite rotation. The after-effect of rotation towards the labyrinthectomised side is stronger than that of the opposite one. Moreover, mechanical or thermal stimulation of the semicircular canals leads to the same results for the vertical canals in these vertebrate classes.

McNally and Tait (frog, 1925), Main (salamander, 1931) and Trendelenburg and Kühn (reptiles, 1908) come to different conclusions. In these animals only rotation in a horizontal plane towards the side of the intact labyrinth and cessation of rotation towards the labyrinthless side called forth labyrinthine responses, when visual orientation was eliminated.

McNally and Tait, in their experiments on the vertical canals of the frog (1933), state emphatically that only a rotation with the ampullary end in advance calls forth response from the vertical canals. Further work in this connection seems to be very desirable.

VI. THE MODE OF ACTION OF THE UTRICULUS AND THE SEMICIRCULAR CANALS.

In the preceding sections it has been shown how scarce and uncertain the knowledge of the function of the different sense endings of the pars superior of the vertebrate labyrinth still is. The enormous divergence of opinion as to the mechanism of the otolith and canal function is therefore not surprising. The experimental basis is as yet much too small to permit a definite pronouncement on this question. Therefore a survey of the various theories will be omitted.

It will have been noticed that throughout this review in describing the function of the sense endings, all implications of functional mechanisms (*e.g.* otolith pressure or tension or possible directions of endolymph currents or pressure) have been avoided. It has sometimes rendered the description less straightforward, but it leaves it free from a theoretical bias, which is unfortunately often found in the literature.

A successful attack on the question of the mode of action will only be possible when the gaps in our knowledge of the function of the different sense endings have been filled by further experimental work.

VII. SUMMARY.

1. The vertebrate labyrinth can be divided into a pars superior, consisting of the utricle and the semicircular canals, and a pars inferior, consisting of the saccule and its various appendages.

2. Only the pars superior is concerned with the maintenance of muscle tone and with reflex reactions to gravity and to linear and angular accelerations. This has been demonstrated for fishes, amphibia and mammals, and, although the evidence is not completely satisfactory, it probably holds for reptiles and birds as well.

The pars inferior takes no part in any of these functions (again with the above reservation as to reptiles and birds), but, even in those vertebrates which lack the organ of Corti, is concerned with sound reception.

Breuer's theory of the localisation of the non-acoustic function of the labyrinth has thus been shown to be erroneous.

3. Attempts have been made to discover which of the receptor endings of the pars superior are involved in each of its functions, by eliminating separately the various endings. The results obtained are not entirely consistent. Production of the static reflexes and of the reflexes to centrifugal force and fast linear acceleration is in all probability the main function of the otolith organ (utricle). It appears, however, that the assumption that the otolith organ is purely static in function is incorrect, for it has been shown that the utricle can be involved in dynamic responses to rotations.

The main function of the semicircular canals is the release of the dynamic reflexes. It has, however, been claimed that the vertical canals take part in the production of *static* reflexes as well.

Both the utricles and the semicircular canals are involved in the maintenance of muscle tone.

4. In the discussion of the general conclusions as to the function of the utricles and of the semicircular canals it is shown that one of the important functional differences between the two receptors consists in their different reaction time, which may be due to the difference in their auxiliary structures and to a different pattern of their nervous connection with the effector organs.

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THE SPECIFIC DYNAMIC ACTION OF PROTEIN AND AMINO ACIDS IN ANIMALS

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I. INTRODUCTION

WHEN food is ingested there is an increase in heat production, oxygen consumption, and carbon dioxide production beginning soon after the ingestion of the food. This is observed also with the hydrolysis products of the primary foodstuffs. This phenomenon, known as the specific dynamic action of food, owes its name to Rubner, who described most of its characteristic energetic features (1902).

The magnitude of the specific dynamic action varies with the substance ingested, with the environmental temperature, and possibly (this point is not yet firmly established) with the nutritional state of the animal. The largest specific dynamic action is that of protein. Thus Rubner found that when 100 calories of food are administered to a warm-blooded animal (in an environmental temperature such

that the "chemical heat regulation" is no longer in operation) the heat produced during the metabolism of this food was approximately 130 calories for protein, 113-115 calories for fat, and 105 calories for carbohydrate. Later experiments indicate that the specific dynamic effects of fat and carbohydrate may be lower than Rubner's figures (Lusk, 1931).

This striking increase in metabolism following the ingestion of protein must have been observed very early. It was known to Lavoisier, and was measured by Bidder and Schmidt (1852). One of the first explanations offered was the obvious one, that increased activity of the intestinal tract—intestinal movements, secretion, digestion and absorption—induced by the ingestion of food is responsible for the increase in metabolism (v. Mering and Zuntz, 1877). This explanation was rendered extremely improbable by the experiment of Rubner (1885), who showed that bones and meat extract do not increase the metabolism of the dog in spite of the intestinal irritation, confirmed by Lusk (1912-13); and by Benedict and Emmes (1912) who found no significant increase in metabolism in men after the administration of cathartics and agar agar. It was finally completely disproven by the observations that intravenously or subcutaneously injected amino acids exert approximately the same specific dynamic action as protein taken by mouth (Table II). Zuntz later (1910) modified the explanation of v. Mering and Zuntz, and held that the increased work of the kidney in excreting the urea and ammonia derived from the ingested protein is responsible for the specific dynamic action of protein. But maximum allowance for increased kidney work will account for less than half of the observed increase in metabolism (Borsook and Winegarden, 1931*a*). This point is considered in more detail below.

Clearly it is necessary to look beyond the intestinal tract for an explanation of the increase in metabolism provoked by protein. The work of the kidney may be a part, but other, quantitatively more important processes intervene before the excretory function of the kidney makes its contribution. This was the one point in common in the opposing explanations offered for the specific dynamic action of protein by the two leading authorities on animal metabolism during the latter half of the nineteenth century (Voit and Rubner). Voit (1881) believed that the rate of intracellular metabolism of apparently all the cells in the body is heightened when the intracellular concentration of protein and its derivatives is increased. His fundamental principle was that the intensity of metabolism of a cell varies with the quantity and quality of the substances brought to it from the blood; hence the variation in the specific dynamic action with different foodstuffs and with the quantity ingested. This conception, opposed by Rubner, was reintroduced by Lusk in a modified form (1912-13), to be abandoned later, and again by Krummacher (1928).

It is more difficult to give a concise statement of Rubner's interpretation of the specific dynamic action of protein. In his *Die Gesetze des Energieverbrauchs bei der Ernährung* (1902), in which most of his work on this problem is collected and summarised, there are only a number of isolated, partial, and not entirely clear statements of his views. The difficulty arises in part from the lack, at that time, of an

accurate or adequate conception of the chemical constitution of the protein molecule and its mode of cleavage in the intestinal canal. Rubner held, in opposition to Voit, that the "intrinsic" metabolism of a normal warm-blooded animal was constant, and was not affected by the concentration of metabolites. He distinguished between "purely thermal" processes and those processes whose energy is utilisable by the organism (p. 380). Yet he also held that carbohydrate was the fuel of preference. He entertained the idea of a calorically significant carbohydrate content (or precursor) in protein. He appears not to have envisaged any use for the deaminised residues of the amino acids (p. 386). The thermochemical losses to the organism, *i.e.* the "purely thermal" processes, must be partitioned between the formation of the sugar moiety, and the disintegration and conversion to urea of the nitrogen-carrying fragments. Though he guessed that the separation of protein into nitrogen-free and nitrogen-containing fractions must entail oxidative processes (p. 385), he believed that the addition of water to protein (by swelling or hydrolysis) and its partial conversion thereby to sugar, may be the source of a considerable evolution of heat (p. 383). A summary of Rubner's views at that time are contained in the following sentence (p. 387): "I believe I have shown one thing, that the theory of sugar formation gives in all its features a simple and not improbable picture of the decomposition (of protein)." Lusk, who enjoyed the privilege of personal discussion with Rubner, gave the following summary of Rubner's views at a later time (Lusk, 1915*a*).

...the fundamental metabolism of a normal warm-blooded animal was always constant and that the effect of food ingestion did not change this. The increased heat production which followed the taking of food was due to heat developed from a lot of intermediary reactions and oxidations and had nothing whatever to do with the fundamental level of the cellular requirement of energy which was entirely unchanged. Thus, when protein was metabolised it could supply energy for the maintenance of true cellular activity in so far as glucose was produced from it, whereas other intermediary cleavage products were simply oxidised with the production of extra heat, which was in no way involved in the life processes of the cell. The utilisation of the energy in protein might be compared with the burning of a tree as fuel for the steam engine, the trunk of the tree being used as fuel within the engine for the production of power, whereas the limbs and twigs are burned as brush outside and supply only heat.

Lusk restated Rubner's hypothesis in more specific terms, and subjected it to experimental test. If Rubner's hypothesis is correct, Lusk argued, the specific dynamic action of glutamic acid should be large, while that of alanine or glycine should be little or nil, since all of the carbon of the last two amino acids, but only three of the five carbons of glutamic acid, are convertible to glucose. Lusk (1912-13, 1913, 1915*a*) found, on the contrary, that alanine and glycine exerted a large specific dynamic action, glutamic acid none. All other observers have obtained relatively large specific dynamic effects with glutamic acid. Lusk's own views regarding the specific dynamic action of protein underwent considerable change. Thus in 1915 he wrote:

The cause of the specific dynamic action of glycocoll and alanine therefore lies in the chemical stimulation of the cells causing them to metabolise more material. This confirms

the older view of Voit that the action of the food increases the power of the cells to metabolise (1915*a*).

Yet in regard to the specific dynamic action of amino acids he differed from Voit. In the same lecture from which the preceding quotation is taken he wrote:

The influx of carbohydrate, of fat, or of alcohol enables the cells to oxidise at a higher level through the increased mass action of food particles available. On the other hand, the metabolism products of glycocoll and alanine may directly stimulate protoplasm without themselves being involved in the oxidative processes and this is called amino acid stimulation.

The evidence which persuaded Lusk most strongly in favour of this view was his observation that the extra glucose recovered in the urine after feeding glycine or alanine to a phlorhizinised dog corresponded to all of the carbon of the amino acids administered. Yet the magnitude of the specific dynamic action was the same as in the normal dog.

In his latest writing (Chambers and Lusk, 1930; Lusk, 1931), Lusk changed his position and inclined to a modified form of one of the clauses of Rubner's explanation: the specific dynamic action represents the energy lost in converting the deaminised residues to glucose. Disagreeing with Rubner he ascribed none of the extra heat to the processes of deamination and urea formation. Aubel and Schaeffer (1932), in accord with this view, proposed a scheme of coupled reactions for the conversion of the deaminised residues to glucose.

The shortcomings of these explanations, apart from the accuracy of the experimental data and questions regarding the thermodynamic validity of the assumptions made, are that they are either not sufficiently precise—those of Voit and Rubner—or that they are too limited. For instance, Lusk, and even more so Aubel, dealt almost exclusively with the conversion of the deaminised residue of alanine to glucose. Phenylalanine, an amino acid which does not lead to an increased glucose excretion in the diabetic animal, exerts a greater specific dynamic action than glycine or alanine. Further, there is no organic connection between the fundamental concepts underlying these explanations and those of other phenomena in protein metabolism.

Recent developments in other fields of protein metabolism and in the theory of the utilisation of the energy of metabolism for physiological chemical work now make it possible to construct at least an outline of a general theory of the specific dynamic action of protein in which the energetic phenomena are analysed in terms of specific reactions, and which forms an integral part of a comprehensive theory of protein metabolism.

In addition to the increase in energy metabolism and urinary nitrogen after the ingestion of protein, there is an increase in urinary sulphur, and a marked rise in urinary uric acid. The increase and decrease in uric acid elimination closely follow the caloric changes in metabolism. The nitrogen and sulphur excretion lag behind. Table I gives some data from an experiment in which the simultaneous changes in these four main variables were observed. Explanations for the specific dynamic action of protein which have been proposed in the past have attempted to account for the changes in not more than two of these variables. It is possible

to deduce from the theory proposed below the relative simultaneous changes in all four variables.

Table I. *Energy metabolism, and urinary nitrogen, sulphur and uric acid following the ingestion of 87 gm. gelatine (Borsook and Keighley, 1934)*

Time	Calories kg. cal. per hour	Urinary nitrogen gm.	Total urinary sulphur mg.	Total urinary uric acid mg.
6.30 a.m.	62.9	—	—	—
6.00-7.10	—	—	—	17.1
8.00	—	—	—	13.5
8.30	77.2	—	—	—
9.00	—	0.60	29.6	35.8
9.30	80.4	—	—	—
10.00	—	0.83	31.8	42.7
10.30	80.5	—	—	—
11.00	—	0.98	36.9	40.9
11.30	75.5	—	—	—
12.00 noon	—	1.14	41.6	36.5
12.30 p.m.	73.4	—	—	—
1.00	—	0.87	38.2	20.9
1.30	70.0	—	—	—
2.00	—	0.78	45.6	20.8
2.30	70.7	—	—	—
3.00	—	0.76	51.1	23.1
3.30	69.8	—	—	—
4.00	—	0.73	49.0	21.1
4.30	70.0	—	—	—
5.00	—	0.65	49.9	13.3
5.30	60.1	—	—	—
6.00	—	—	—	19.2

II. STIMULATION OF ENDOGENOUS METABOLISM?

(1) THE GENERAL METABOLISM

The central problem is the analysis of the increase in caloric metabolism in terms of chemical reactions. Such an analysis would be impossible, indeed irrelevant, if it be true that an increased concentration of amino acids in the tissues induces a general stimulation of the cells without the amino acids themselves necessarily being oxidised—the so-called stimulation of endogenous metabolism. The main evidence adduced in recent times in support of this view is the increase in urinary uric acid (Lewis, Dunn and Doisy, 1918), and sulphur (Kiech and Luck, 1931-32) following the administration of amino acids; and the observation of Rapport and Katz (1927) that there is an increase in metabolism following the addition of glycine to the circulating blood of an isolated hind-leg preparation.

The experiment of Rapport and Katz is open to the criticism that the concentration of glycine in the circulating blood was relatively enormous, about 1 per cent. Bornstein and Roese (1929-30) observed no increase in oxygen consumption when glycine (concentration not given) was added to the blood perfused through the extremities of a dog, while a definite increase in the oxygen consumption

of the isolated liver was observed when glycine was added to the perfusing blood. Nothaas and Never (1930), with 0.1 per cent. glycine in the perfusing blood, confirmed both observations of Bornstein and Roese. Further evidence against the idea of a general stimulation of the tissues is the observation of Bornstein and Roese that the oxygen consumption of the extremities was not increased with glycine added to the perfusing blood even when the liver was in circuit with the extremities. The significance of the observation of Rapport and Katz is lessened also by the observations of Needham (1930) that neither glycine nor alanine increased the respiration of a minced muscle preparation, though a marked increase was observed with glutamic acid.

Evidence from other sources is also decisively against this explanation. The increased heat production in a dog after a large meat meal may last for 20 hours (Williams, Riche, and Lusk, 1912). Yet Wishart (1915) found the non-protein nitrogen of the muscles essentially unchanged 6 hours after a meat meal, and concluded from her data that the specific dynamic action of protein cannot be ascribed to a stimulation of the endogenous metabolism of all the tissues. Van Slyke and Meyer (1913-14) found 4 hours after the intravenous injection of a mixture of amino acids in dogs that the amino acid concentration of the liver was subnormal. After hepatectomy glycine and alanine exert no specific dynamic action (Wilhelmj, Bollman, and Mann, 1928). Similarly Dock (1931) demonstrated that the hind-quarters participate very little, if at all, in the increased oxygen consumption during protein metabolism. 80 per cent. of the increase in metabolism could be ascribed to the viscera—liver, kidneys, and intestine.

The caloric data expressed as a ratio of excess calories to excess nitrogen indicate that as far as the general metabolism is concerned the reverse of a stimulation of endogenous metabolism occurs. There is, in effect, a sparing action. If there is any considerable general stimulation of endogenous metabolism the increase in calories over the basal, per gram of extra nitrogen metabolised, should always be definitely greater than the physiological heat value of the protein or amino acid used (regardless of whether the immediate origin of the nitrogenous material metabolised was the cells or the food). The increase in the general metabolism would be added to the physiological heat of combustion of the protein. The data in Table II show that, except in the case of a starving animal, the values are always much less, one-half, and even one-third, of the physiological heat value of the nitrogen ingested. Obviously the carbon of the ingested protein or amino acid is not all oxidised, or if it is, some of the basal carbon is spared from oxidation. This could be described as the carbohydrate (or fat) sparing action of protein, the converse of the classical protein-sparing action of carbohydrate. In view of this sparing of tissue carbon a stimulation of general endogenous metabolism as an explanation for the increase in metabolism loses all its force. Clear examples of this sparing action are to be found in the well-known experiments of Rubner (1902, pp. 347-55) on the feeding minimum where the energy requirements of dogs over a range of external temperatures from nearly zero to body temperature were supplied solely by meat. In these experiments there was a complete

sparing of the animal's tissues. The general endogenous metabolism could hardly have been stimulated.

If the endogenous stimulation theory be true it would be reasonable to expect that the specific dynamic action of protein would be greater in a well-nourished animal well stored with reserves of glycogen and of protein than in a starving animal. If anything, the reverse is the case (Wilhelmj and Mann, 1930; see below).

A variant of the stimulation of endogenous metabolism hypothesis is the so-called "pharmacodynamic" stimulation, in which the increase in metabolism following the ingestion of protein is ascribed to an increased activity of the ductless glands. This explanation is based on observations such as those of Liebeschütz-Plaut (1922, 1925), that in cases of *dystrophia adiposogenitalis* there is a reduction in the specific dynamic action of protein. This was confirmed in men by Liebesny (1924), and by Foster and Smith (1926) in hypophysectomised rats. Nothaas (1929) found the specific dynamic action of protein moderately increased in rats after feeding anterior pituitary extract for several weeks; and Nothaas and Never (1930) observed a greater increase in the oxygen consumption of the isolated liver perfused with blood when a high concentration of anterior pituitary extract was added before the glycine to the perfusing blood than when glycine alone was added.

There was, and still is, some confusion here between rate of metabolism of protein and the specific dynamic action of protein expressed as an absolute value per gram of protein metabolised. In none of the above-mentioned experiments were any measurements made of the amount of protein metabolised during the interval through which the energy metabolism was observed. From experiments in which the nitrogen also was measured it appears that a slower rate of metabolism of protein was probably responsible for the low specific dynamic action observed in this and other glandular dystrophies. For instance, the ratio of excess calories to excess urinary nitrogen is of a normal order of magnitude in thyroidectomised animals, whether phlorhizinised or not (Dann, Chambers and Lusk, 1931-2), and in hypophysectomised animals (Gaebler, 1929). In the case of the thyroidectomised animal the results are particularly striking because phlorhizin no longer increases the basal metabolism, nor is there the same increase in basal nitrogen metabolism. There doubtless are hormonal influences on the rate of protein metabolism as on that of other metabolites. In pituitary and in thyroid disorders these rates may be above or below normal according to the nature of the disturbance. If the rate of metabolism of ingested protein is subnormal the hourly increase in metabolism, *i.e.* the hourly specific dynamic action in the first hours after ingestion, will be subnormal. Whether there may be significant and characteristic differences in the absolute values of the specific dynamic action, *i.e.* in the ratio of excess calories to protein metabolised, in different pathological conditions is another question, on which unfortunately the very large volume of work on the specific dynamic action of protein in various clinical endocrine disorders throws no light, chiefly because the energy metabolism only, without the concomitant changes in urinary nitrogen, was measured. The data on experimental animals in which the nitrogen excretion was measured indicate as stated above that the

endocrines do not influence significantly the absolute value of the specific dynamic action of protein. Based on changes in energy metabolism alone it has been stated that the specific dynamic action of protein is of a normal order of magnitude in patients with exophthalmic goitre (Dubois, 1916), in hypophysectomised animals (Braier, 1932; Mazzocco, 1933), in patients with pituitary disease (Fulton and Cushing, 1932; Johnston, 1932; Dagg and Eaton, 1933), and in adrenalectomised dogs (Nord and Deuel, 1928).

Stimulation of the central nervous system has been suggested by Guttmacher and Weiss (1927) as the mechanism for the specific dynamic action of protein. Their experiments were carried out on rabbits. They observed an increase in oxygen consumption after the administration of glycine or glucose when their animals were under light urethane narcosis (nervous reflexes still present), but were unable to produce any with these substances when the animals were in deep narcosis (no visible reflexes). It is not possible to interpret these observations because no urinary nitrogen data are given. The doses of glycooll given were enormous, for instance 17.5 gm. of glycine to an animal weighing 1950 gm., and judging by the observations of Lewis and Luck (1933) on rats were probably toxic. In any case the conditions here are not comparable to those in experiments in other warm-blooded animals, since 20 gm. of glucose were as effective in increasing the oxygen consumption as 10 gm. of glycine (see below).

(2) THE NITROGEN METABOLISM

The increases in urinary uric acid and sulphur after the ingestion of protein remain to be accounted for, if the explanation of a general stimulation of the tissues is untenable. It is possible that these may represent a stimulation, if not of the general metabolism of the tissues, at least of the endogenous nitrogen metabolism. This was the interpretation which Lewis, Dunn, and Doisy (1918) placed upon their observation that the urinary uric acid is increased in men for some hours after the ingestion of amino acids; and by Kiech and Luck (1931-2) on their finding in rats of an increase in urinary sulphur after the injection of neutralised aspartic acid.

Borsook and Jeffreys (1935) found that the addition of amino acids does not accelerate tissue protein katabolism when slices of kidney, liver, spleen, and sections of nearly intact diaphragm and intestine are exposed to increased concentrations of amino acids. Earlier it was shown (Borsook and Keighley, 1934) that the rise in urinary uric acid is probably the result of an increased formation of uric acid from ammonia arising from the oxidative deamination of the products of digestion. This process is most rapid in the first few hours after a meal when the concentration of amino acids in the tissues and hence their rate of deamination are highest. It may be expected therefore (see below) that the rise and fall in energy metabolism will parallel the rise and fall in oxidative deamination, and hence from the foregoing the synchronous rise and fall from hour to hour of the curves of oxygen consumption and urinary uric acid.

The administration of proteins and amino acids is not invariably followed by an increased excretion of uric acid and allantoin, as it is by an increase in energy metabolism. Christman and Lewis (1923) found that the feeding of glycine, alanine, glutamic acid, or urea to rabbits was followed by a marked decrease in the daily elimination of allantoin. Similar results, though less striking, were obtained with gelatine. In men, after the ingestion of glycine, there is, at times, no increased elimination of uric acid (Borsook and Keighley, 1935), and there may even be a decrease. The rise in urinary uric acid following the ingestion of a non-purine-containing protein such as gelatine, or a single amino acid, is not therefore in itself evidence of a stimulation of tissue nitrogen metabolism. A stimulation of nitrogen metabolism alone by amino acids is all the more improbable in view of the absence of any stimulation of the general metabolism of the tissues.

Table I shows the rise in urinary sulphur following the ingestion of gelatine. It is very difficult to separate the urinary sulphur following the ingestion of protein into endogenous and exogenous fractions because all proteins contain more or less sulphur. The N/S ratio of the gelatine used in Table I was 15. It seemed that a clearer picture might be obtained by observing the changes in urinary sulphur following the ingestion of non-sulphur-containing amino acids, and of haemoglobin, a protein low in sulphur, which induce an increase in metabolism and a rise in urinary uric acid similar to (but not necessarily quantitatively the same as) that of normal protein. A large number of such experiments were performed by Borsook and Keighley (1935). The results were irregular. In one experiment with glycine the hour by hour urinary sulphur figures showed a short but definite rise. More often with glycine and with alanine no change was observed. Over the 24-hour period the sulphur excretion on a given day was very nearly the same whether no nitrogen was ingested, or whether the nitrogen balance was maintained by ammonia, a single amino acid (glycine or alanine), a mixture of amino acids, or a protein such as haemoglobin. The evidence of the sulphur figures indicated that there is no stimulation of endogenous nitrogen metabolism over the whole period.

These and other experiments left the impression that the sulphur excretion over a short period is affected more by other factors, such as disturbances in the acid base balance, than by an increase in the concentration of amino acids in the tissues. For instance, there was always a definitely greater excretion of urinary sulphur when sodium bicarbonate was taken on a fasting day than during a fast without bicarbonate. This, possibly, is the explanation for the increase in urinary sulphur after the injection of neutralised aspartic acid observed by Kiech and Luck (1931-2). The excretion of the base, after the metabolism of the carbon and nitrogen of the amino acids, carried with it some additional sulphur.

The observations of Reid (1934) may be cited in this connection as illustrating the difficulty of interpreting the changes in sulphur excretion. 22.5 gm. of glycine per square metre of body surface were fed to normal and schizophrenic men. In the normals the sulphate excretion was not increased in the first 6 hours over the initial "pre-glycine" period. In the fasting controls, with water or urea to induce diuresis, the excretion was only 70 per cent. of the initial period. Breakfast (details

not specified) also maintained the sulphate excretion at the initial level. Glycine added to the breakfast increased the excretion 6–10 per cent. The glycine induced a decrease in blood sugar of 10–15 mg. per cent. for 2–3 hours, followed by a rise to the initial fasting level.

From the observation of Chaikoff and Larson (1935) that insulin and epinephrine increase the uric acid and allantoin excretion in dogs, and the observation of Nord (Nord and Deuel 1928), that glycine provokes in rabbits an increased production of epinephrine it might appear that glycine at least exerts its specific dynamic action through stimulation of the adrenal medulla. Against this interpretation are the observations cited above, that glycine reduces the allantoin excretion in rabbits (Christman and Lewis, 1923), and that the specific dynamic action of glycine is the same in adrenalectomised as in normal dogs (Nord and Deuel, 1928).

If there is a stimulation of endogenous metabolism after the ingestion of protein or amino acids, in an animal in nitrogen balance a compensatory depression must occur later to make good the earlier increased endogenous losses. This depression is all the more necessary if the bulk of the nitrogen metabolised in an animal in nitrogen balance is taken to be of exogenous origin. A compensatory decline is observed as late as the following 24-hour period after nitrogen metabolism is stimulated by insulin (Chaikoff and Larson, 1935). These authors in this and in subsequent papers drew attention to the increase in excreted uric acid and allantoin in normal and in Dalmatian dogs following the administration of insulin. This they showed could be ascribed to the increased epinephrine production evoked by the low blood sugar brought on by the insulin. In every case in the period succeeding the insulin or epinephrine period the total nitrogen was below average, though the uric acid or allantoin excretion was not depressed. There is here a clear pharmacodynamic stimulation of nitrogen metabolism. It is followed by a compensatory depression. Gigon (1911) observed such a depression after the ingestion of 50 gm. of casein—not with larger quantities—both in the energy metabolism and nitrogen excretion. He quoted a number of earlier observations of a similar depression in metabolism after the ingestion of small quantities of protein. There is no record in any of the later work of such a depression (Williams, Riche and Lusk, 1912; Csonka, 1915; Lusk, 1915*b*; Strang and McClugage, 1931). Numerous experiments in this laboratory (Borsook and Keighley, 1935), in which observations were extended for 10 hours after the ingestion of amino acids and different quantities of protein, gave no evidence of a depression below the fasting values for the same time of day either in the hourly caloric metabolism, total urinary nitrogen, uric acid or sulphur excretion.

III. THE CORRELATION BETWEEN SPECIFIC DYNAMIC ACTION AND THE QUANTITY OF PROTEIN OR AMINO ACID METABOLISED

With stimulation of the general metabolism of the tissues excluded the path is clearer for an analysis of the specific dynamic action of protein and amino acids in terms of specific reactions involving the amino acids and the products of their

metabolism. Further, with this exclusion of a general stimulation of the tissues, there is no reason to expect different energy changes whether tissue protein and amino acids or amino acids immediately derived from ingested protein are metabolised.

(1) THE ENERGY COST OF SYNTHESIS AND STORAGE OF PROTEIN

Before the specific dynamic action of protein can be related to the quantity metabolised two other possibilities must be considered, the energy cost in the synthesis of protein, and the possibility of an increased metabolism as a consequence of the presence of more protein in the cells, *i.e.* of the increase in "protoplasm" as it were, when protein storage occurs. These possibilities might have been passed over without any consideration if the protein metabolised in any one day were entirely (after making a small allowance for the replacement of the "wear and tear" losses) of exogenous origin, *i.e.* derived from the protein ingested on that day. But in man, for instance, in nitrogen equilibrium, more than half the ingested protein is probably synthesised into protein or polypeptides (Borsook and Keighley, 1935). It is improbable that the increased metabolism in peptide synthesis exceeds 4 calories per gram of nitrogen—the maximum allowance for the synthesis of urea from ammonia (see below). Rubner (1902, p. 256) and Hoobler (1915) observed no increase in metabolism as a result of the storage of nitrogen. It appears, therefore, that even when a considerable synthesis and storage of protein is accepted as a normal occurrence these processes may be omitted in the first approximate accounting of the specific dynamic action of protein.

(2) THE PROBLEM OF ESTIMATING THE METABOLISM OF THE INGESTED PROTEIN OR AMINO ACID

There appears to be no alternative but to relate at least the largest fraction of the specific dynamic action to the quantity of protein or amino acid metabolised. On this point all later students of this subject are agreed (Lusk, 1915*b*; Terroine and Bonnet, 1926; Aubel, 1925, 1927, 1928; Aubel and Schaeffer, 1932; Wilhelmj and Bollman, 1928; Borsook and Winegarden, 1931*b*; Wilhelmj, 1934). The increase in metabolism may be referred to the weight, or nitrogen, or physiologically available energy content of the protein or amino acid metabolised. The problem of measuring the quantity of protein or amino acid metabolised then arises. This problem was the more difficult because of an unnoticed contradiction between the prevailing theory of protein metabolism and a fundamental assumption usually made in computing the results in experiments on the specific dynamic action of protein. This assumption is that the exogenous quota is superimposed on the initial or basal protein metabolism which continues at the same level throughout the experiment. But this basal metabolism is much greater than the endogenous metabolism (in Folin's sense). It consists for the most part of continuing metabolism (Borsook and Keighley, 1935). Yet it is assumed that most of the nitrogen excreted, when protein or an amino acid is fed, is of exogenous origin, only a small quantity being retained to replace the

endogenous (not basal) losses. If this is the case then most of the basal nitrogen metabolism is suspended after the ingestion of protein, and it is incorrect to subtract the basal value at the beginning of the experiment multiplied by the number of hours through which the experiment has been continued from the total nitrogen excreted in this period, and to consider this difference as representing the ingested protein or amino acid nitrogen which has been metabolised. In the case of protein it is immaterial, as far as the energy changes are concerned, whether ingested or tissue protein has been metabolised. With individual amino acids the situation is different because different respiratory quotients must be assigned to the urinary nitrogen derived from different amino acids, which in turn affect the energy coefficients to be applied to the oxygen consumption and carbon dioxide production in computing the metabolism by indirect calorimetry. If the prevailing theory of protein metabolism is accepted then most of the nitrogen in the urine is of exogenous origin; it is derived from the nitrogen ingested. The total nitrogen over a 24-hour period, for example, is not the sum of ingested nitrogen plus 24 times the hourly initial or basal nitrogen, since the latter has been largely suppressed. On the other hand, if the basal or "continuing" metabolism (Borsook and Keighley, 1935) is considered as persisting throughout, then only the urinary nitrogen in excess of this value is of exogenous origin. This excess in a 24-hour period may be less than half the quantity of nitrogen ingested. The remainder is stored as labile (polypeptide or protein) nitrogen. On this basis the determination of the proper respiratory and energy coefficients to be applied to the excess nitrogen fraction of the oxygen used and carbon dioxide produced is a separate problem in each case and often can only be guessed at. If all of the carbon arising from the same source as the excess nitrogen is burned then the coefficients are those of the protein or amino acid fed. But this is rarely the case. The various complications which may occur are discussed in a later section. Fortunately, in many cases, the final value of the metabolism is nearly the same with widely divergent methods of computation.

(a) *The "extra-glucose" method*

A decision on this question is a prerequisite for an internally coherent analysis of the specific dynamic action of protein. Lusk evaded this issue and based his estimates of the quantity of amino acid metabolised in a given interval on the increased excretion of glucose by phlorhizinised dogs after the administration *per os* of glycine and alanine (Csonka, 1915). The calculations whereby the basal glucose and the glucose derived from the ingested amino acid were estimated cannot be accepted without important reservations. For example there are uncertainties in the basal glucose excretion in these animals; at times the hourly glucose excretion was less in the 4 hours following the termination of the period during which the extra glucose excreted was considered as arising from the ingested amino acid, than in the preceding periods, or some hours afterwards. The increased metabolism, and hence combustion of glucose, as a consequence of the specific dynamic action of the amino acid, was ignored; the nitrogen-sparing action of the amino acids demonstrable in Csonka's data, and also the quantity of amino acid which escaped

in the urine unmetabolised, were not taken into account. Even accepting Csonka's conclusions regarding the fraction of the administered amino acid carbon converted to glucose in the phlorhizinised dog in his experiments, it remained to be proven that the rate of metabolism in the normal dog is the same as in his phlorhizinised animals. Lusk, apparently, took this for granted. Yet his data show large differences. In two experiments with 20 gm. of alanine the extra nitrogen in the urine amounted to 57 and 65 per cent. of the alanine nitrogen fed, the corresponding amounts of urea and ammonia nitrogen to only 35 and 44 per cent. respectively. In a similar experiment on the phlorhizinised dog, which was the basis of Lusk's calculations, the extra nitrogen in the urine over the same period amounted to 84 per cent. of the alanine nitrogen fed. There is another difficulty in accepting Lusk's calculations and interpretation. The specific dynamic action, according to Lusk, is the increase in metabolism attending the conversion of the deaminised residues to glucose. The extra glucose formed (and excreted) in the phlorhizinised dog, or the extra nitrogen in either animal, would serve therefore, all other factors being equal, as an index of the amount of the amino acid metabolised in both the normal and the phlorhizinised dog. Lusk preferred the extra glucose—hence the experiments of Csonka—because the glucose is excreted sooner. But if the fundamental hypothesis here is correct the increase in metabolism should not lag behind the excretion of the glucose. Yet this occurs in the phlorhizinised dog, as Lusk admitted. This result favours the suggestion made by Terroine and Bonnet (1926) and later by Borsook and Winegarden (1931*b*) that some or all of the extra glucose excreted may represent not glucose formed from the deaminised residue of the amino acid, but the sparing of tissue glucose by the combustion of the deaminised residue. The extra-glucose method of estimating the amount of protein or amino acid metabolised, as well as explanations of the specific dynamic action of protein based upon the hypothesis of a necessary conversion to glucose of deaminised residues, break down completely with ketogenic amino acids. Tyrosine and phenylalanine exert a larger specific dynamic action than glucogenic alanine or glycine (Terroine and Bonnet, 1926; Rapport and Beard, 1927; Wilhelmj and Bollman, 1928).

(b) *The "extra-nitrogen" method*

A simple and direct expression of the specific dynamic action is as a ratio of the increase in metabolism over the basal to the urinary nitrogen in excess of the basal. The use of this method requires that a decision be made on the interpretation of the urinary nitrogen as discussed above. Wilhelmj and Bollman (1928) were apparently the first to state explicitly that "the most suitable manner of expressing the relationship between specific dynamic action and an administered amino acid is as calories per millimol of amino acid deaminised". The chief difficulty and objection to this method is that the nitrogen excretion lags behind the increase in metabolism. This method would appear then to be inapplicable to those experiments, which are the majority, in which the recorded observations were terminated before either the energy metabolism or the nitrogen excretion had returned to the basal level. It is not a practical criticism of this method that the observations are usually terminated

before all of the ingested nitrogen is accounted for as excess urinary nitrogen. Even over a 24-hour period the excess urinary nitrogen is less than the ingested nitrogen. In spite of the fact that practically all of the studies on specific dynamic action are short-period experiments, and in spite of the lag in the urinary nitrogen excretion a significant correlation does exist between the observed increase in energy metabolism and the excess urinary nitrogen (Figs. 1 and 2) (Borsook and Winegarden, 1931*a*).

This correlation was not accepted by Lusk and his pupils, nor by Aubel and Schaeffer (1932). Their criticism is derived from their refusal to ascribe a significant

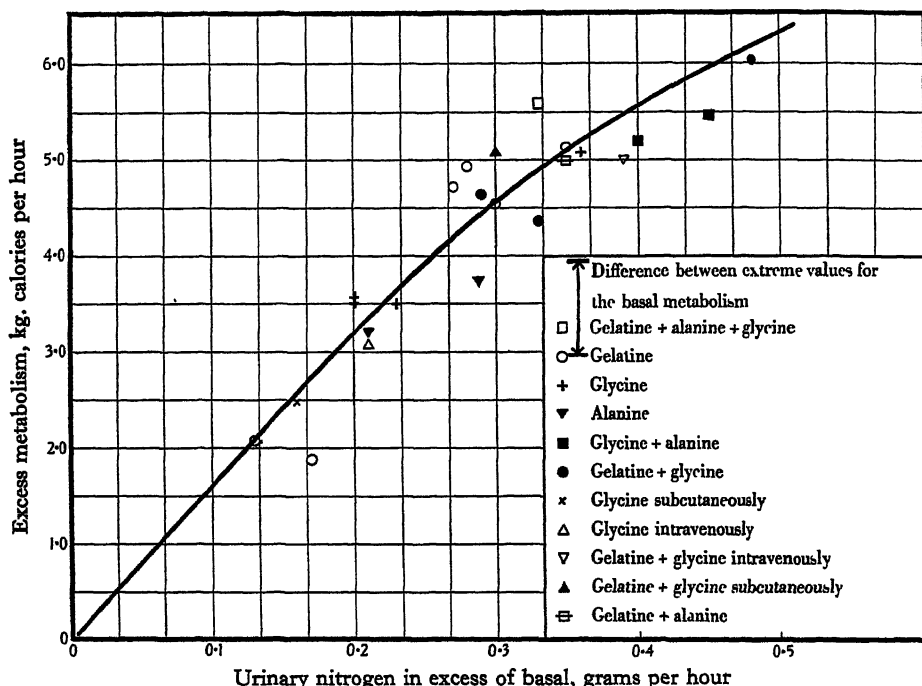


Fig. 1. The relation between the excess urinary nitrogen and the specific dynamic action of alanine, glycine, and gelatine (Weiss and Rapport, 1924).

fraction of the specific dynamic action to the metabolism and excretion of the nitrogen. Aubel and Schaeffer gave the following reasons. In any one experiment the curves of extra-urinary nitrogen and extra metabolism are not synchronous. The individual points in Figs. 1 and 2 are averages, each point representing an experiment lasting 4–6 hours; this correlation is not demonstrable if the values for each hour are plotted. Finally in the experiments of Rapport and Beard (1927, 1928) the observed change in metabolism with different amino acids bears no relation to the extra-urinary nitrogen. Analysis of the data upon which this criticism is based shows that none of these points can be sustained (Borsook, 1935*b*). A brief review will be cited here. The advantage of and the justification for taking the data for the first 4–6 hours is that after the first 4 hours a state is attained

in which the increase in calories and the increase in urinary nitrogen over the basal run nearly parallel. Since only a small fraction of the nitrogen is excreted in the first 2 hours, much more in the next 2-4 hours in which the peaks of metabolism and nitrogen are attained, the values for the ratio excess calories/excess nitrogen are not significantly different (with amino acids, gelatine, and moderate amounts of meat) for the short interval or for the whole period of the experiment. For instance, in the experiments of Gigon (1911) which Aubel and Schaeffer cited the

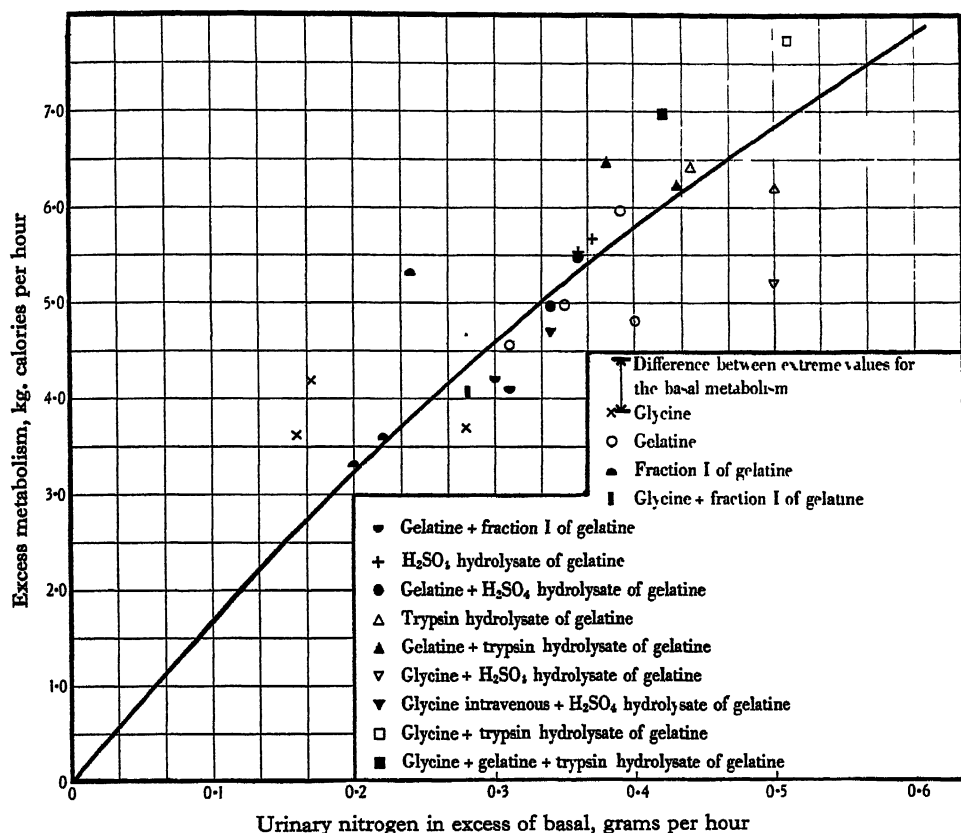


Fig. 2. The relation between the excess urinary nitrogen and the specific dynamic action of glycine, gelatine, and gelatine hydrolysates (Rapport and Beard, 1927, 1928; Rapport, 1926-7).

values of this ratio were 17, 14, 21, and 19 respectively, when 50, 100, 150, and 200 gm. of casein were ingested, and the period of observation varied from 3.5 to 10 hours. Furthermore the nitrogen excretion does not lag far behind the changes in energy metabolism. In the experiment of Gigon with 50 gm. of casein which Aubel and Schaeffer cited as an example of the nitrogen excretion continuing long after metabolism had returned to normal, the data show that in the interval from 3.5 to 5 hours after the ingestion of the casein the nitrogen excretion had returned to the basal level, and was actually below the basal level

accompanying in this respect the energy metabolism, during the next 5 hours. The reason for not considering the individual hourly values in any one experiment is that the hourly caloric as well as nitrogen data are irregular. It is obvious, however, that if the averages in different experiments group themselves about a smooth curve the individual values from which these averages were obtained will also. In the terminal hours of an experiment the increased metabolism represents only (or mainly) the cost of excreting the urea. This is a small fraction of the total specific dynamic action (see below), and where moreover the extra nitrogen is small, as it must be here, the expected increase in metabolism representing the cost of excreting the nitrogen is so small as to fall within the variations of the basal metabolism. It is for this reason that the most reliable values for the total specific dynamic action are obtained during the steady state. The data of Rapport and Beard with different amino acids which Aubel and Schaeffer cited are not discordant if the differences in the metabolism of these amino acids is taken into account. It is to be expected (see below) that the specific dynamic action of tyrosine and phenylalanine will be greater than that of glycine or alanine, for example. In the case of arginine urea arises by simple hydrolysis, and in the case of histidine by splitting of the imidazole ring without oxidation. The items for oxidative deamination in the specific dynamic action will be missing with arginine and histidine, and for urea formation also in the case of arginine. In spite of the fact that no specific dynamic action was assigned to histidine and valine by Rapport and Beard and by Aubel and Schaeffer the data show that increases in metabolism which were observed were more than sufficient to account for the cost of excreting the nitrogen.

By ignoring the urinary nitrogen, except for purposes of indirect calorimetry, Lusk and his pupils gradually moved toward the position that the different increases in energy metabolism which they observed in their relatively short periods of observation with different substances are to be ascribed to constant, specific, and absolute differences in the calorigenic potentialities of these substances; not at all to differences in their rates of metabolism. Hence they expressed their results in terms of the total quantity administered, in spite of the fact that their periods of observation were short, and that Lusk was the first to adopt the view that the increase in metabolism was to be related to the quantity metabolised. They paid little or no attention to the probability that if two substances are compared for their specific dynamic effects, glycine and glutamic acid for example, one of which is absorbed and metabolised quickly, the other very slowly, and the data upon which the comparison is based are derived from observations during the first few hours, the substance metabolised more quickly, all other factors being the same, will appear to exert the larger specific dynamic effect if the comparison is referred to the amount ingested. Interpreting their data as rates and expressed in the form of a ratio of excess calories to excess nitrogen, phenomena which Lusk (1928) found anomalous present no difficulty, viz. the apparent "neutralisation" of the specific dynamic effects of glycine and alanine when administered with protein (Weiss and Rapport, 1924), the high specific dynamic effect of gliadin (which

consists of more than 40 per cent. glutamic acid) in view of the apparently low or absent specific dynamic effect of this amino acid when administered alone (Rapport, 1924). Similarly the apparently low specific dynamic action of protein in cases of endocrine disturbance, when they occur, are frequently no longer exceptions calling for explanation of the small increase in metabolism. The problem is the description of the changes in mechanism responsible for the subnormal rate of metabolism of the protein.

The usefulness of expressing the specific dynamic action as a ratio of excess calories to excess nitrogen is exemplified in the uniformity of the results so expressed of a great number and variety of experiments. Table II contains nearly all the data on the specific dynamic action of protein and amino acids from the literature available here, recalculated on this basis. Data excluded are one set of values by Terroine and Bonnet (1929) in an experiment where very large doses of alanine and glycine were injected into rabbits; those of Rubner where the temperatures were not the same on the experimental and fasting days; and most of the short-period experiments of Rapport and Beard (1927, 1928) where there was too little extra nitrogen excreted. Lewis and Luck (1933) have carried out an extensive investigation of the calorogenic effect of increasing amounts of different amino acids on rats. Only the caloric data for glycine and glutamic acid have been published so far. They promise to present later a discussion of the relation between the nitrogen excretion and the increase in metabolism.

IV. A THEORY OF THE SPECIFIC DYNAMIC ACTION OF PROTEIN

The upper and lower limits of the data in Table II, and the abnormal values, are accounted for by the theory that the increase in metabolism is a composite of two groups of factors, one of which is nearly constant, and the other variable. The constant factor comprises the increased oxygen consumption attending the oxidative deamination of the amino acids. In this process one molecule of oxygen is used for every molecule of nitrogen deaminised. In addition there will be an uncertain quantity of oxygen used in the conversion of ammonia to urea not exceeding another molecule of oxygen per molecule of urea nitrogen (Borsook and Keighley, 1934). Of the variable fraction one part is the increase in metabolism attending the increased excretion of nitrogen, the other, the more important fraction, is the metabolism of the deaminised residues (Borsook and Keighley, 1934).

Two of the fundamental postulates of this theory have already been discussed, that there is no stimulation of the general metabolism of the tissues by the mere presence of amino acids in increased concentration, and the basal metabolism of nitrogen continues undiminished after the ingestion of protein or amino acids. A third postulate concerns the utilisation of the energy for physiological work. It has been discussed in detail elsewhere (Borsook, 1935*a*). Briefly it is that in the reaction $A+B \rightarrow C+D$, where A is a metabolite and B is oxygen, none of the energy of this reaction is available for physiological work when B combines directly with A . Hence all the energy of oxidative deamination, for example, is lost as far as the performance of physiological work is concerned. The only means whereby work

Table II. *Specific dynamic action of protein and amino acids.*

Observer	Substances administered	Environmental temperature ° C.	Excess calories Excess nitrogen Cal./gm. ¹	Remarks
Rubner (1902)	Meat	15 15.3 20.2 33	8 5 9, 12 13, 15, 17	Dog
Gigon (1911)	Casein	?	17, 14, 21, 19	Man
Lusk (1912-13)	Glycine Alanine	?	16, 14 16	Dog
Williams, Riche and Lusk (1912)	Meat	26-27	11, 13	Dog
Lusk (1915)	Glycine Alanine	27°?	18, 15, 16 17, 19	Dog Dog, phlorhizinised
Rapport (1924)	Beef Casein Casein + HCl Gliadin Codfish Chicken	25-26	19 29, 27 25 21, 20 17 22	Dog
Weiss and Rapport (1924)	Beef, 200 gm. Beef, 400 gm. Beef, 600 gm. Beef, 800 gm. Gelatin + glycine + alanine Gelatin Glycine Alanine Glycine + alanine Gelatin + glycine Glycine Glycine Gelatin + glycine Gelatin + glycine Gelatin + alanine	25-26	13 10 9 8 17 16, 11, 17, 18, 15, 15 18, 18, 15, 14 15, 13 13, 12 16, 13, 13 16 15 13 17 14	Dog Subcutaneous Intravenous Intravenous Subcutaneous
Terroine and Bonnet (1926)	Glycine, alanine, aspartic, glutamic, valine, leucine, cystine, and lysine	15	8 each	Frog, injected 14 mg. NH ₂ N per frog. Weight not given
	Tyrosine and phenylalanine	15	9 each	
	Tryptophane and histidine	15	10 each	
Rapport and Beard (1927, 1928)	Glycine Gelatin Fraction 1 of gelatin Glycine + fraction 1 of gelatin H ₂ SO ₄ hydrolysate of gelatin Gelatin + H ₂ SO ₄ hydrolysate of gelatin Tryptic hydrolysate of gelatin Gelatin + tryptic hydrolysate of gelatin Glycine + H ₂ SO ₄ hydrolysate of gelatin Glycine + H ₂ SO ₄ hydrolysate of gelatin Glycine + tryptic hydrolysate of gelatin Glycine + gelatin + tryptic hydrolysate of gelatin Phenylalanine	25-26	23, 26, 14 15, 14, 15, 12 17, 16, 22 15, 14, 13 15, 15 15, 15 15, 12 18, 15 10 14 15 17 44, 40	Dog Intravenous

¹ The values for the metabolism used here are in every case those given by the author, even where the mode of calculation by indirect calorimetry is open to question.

Table II (cont.)

Observer	Substances administered	Environmental temperature ° C.	Excess calories Excess nitrogen Cal./gm.	Remarks
Wilhelmj and Bollman (1928)	Glycine Alanine Phenylalanine	25-30	35, 29, 20, 21 22, 28 56, 44	Dog, intravenous Dog, intravenous Dog, intravenous, 0.1 gm. $\text{NH}_2\text{N/kg}$.
Gaebler (1929)	Beef heart, 200 gm. Beef heart, 400 gm.	?	15 14	Dog, normal Dog, hypophysectomised
Wilhelmj and Mann (1930)	Glycine Alanine	25-30	13, 14, 19, 16, 16 24 59*, 48†, 41‡ 18, 12, 11, 11, 8 113§, 34 , 34¶	Dog, normal Dog, starved Dog, starved Dog, normal Dog, starved, intra- venous; 0.1 gm. $\text{NH}_2\text{N/kg}$.
Dann, Chambers and Lusk (1931-32)	Meat	26-27?	17 17, 18, 14, 9 12, 10, 28, 16, 16	Dog, normal Dog, thyroidectomised Dog, thyroidectomised + phlorhizinised
Wilhelmj, Bollman and Mann (1931)	Glycine Glycine	?	19, 18, 16 16, 16	Dog, intravenous Dog, <i>per os</i>
Borsook and Keighley (unpublished experiments)	Meat, 315 gm. Meat, 150 gm. Meat, 315 gm. Meat, 450 gm. Meat, 630 gm. Gelatine, 33 gm. Gelatine, 69 gm. Gelatine, 87 gm.	23 23	28 29 18, 25 28 21 28 18, 18, 20, 31 24	Man, poorly nourished Man, normal state of nutrition Man, normal state of nutrition Man, normal state of nutrition Man, normal state of nutrition Man, normal state of nutrition Man, normal state of nutrition Man, normal state of nutrition
Wilhelmj (1934)	Glycine	?	10, 11, 11, 11, 11, 12, 12, 13, 13, 13, 14, 14, 14, 15, 15, 16, 16, 16, 16, 22	Dog, intravenous

* 75 per cent. of administered glycine not accounted for.

† 74 per cent. of administered glycine not accounted for.

‡ 71 per cent. of administered glycine not accounted for, injected with 50 c.c. 25 per cent. glucose.

§ 85 per cent. of administered alanine not accounted for.

|| 74 per cent. of administered alanine not accounted for.

¶ 71 per cent. of administered alanine not accounted for.

can be obtained from the above reaction is when *A* and *B* are separated and the electron transfer is effected by chemical intermediaries or by electron conduction through the cell.¹ The electrons then pass from *A*, a metabolite, through some other

¹ Chemical work could be performed by "activated" molecules arising from the direct combination of oxygen with a metabolite. This is a third possible mechanism. Nothing is known at present regarding the "life time" and reactivity of activated organic molecules in aqueous solutions. For this reason and also because all the physiological energetic phenomena (other than photochemical) known to the author seem to point away from "activation" processes a detailed discussion of this possible mechanism has been omitted.

system on which work is performed before arriving at *B*, cytochrome, for example, where they participate in the combination with oxygen and formation of water. The performance of work at the cost of the combustion of metabolites makes it necessary to postulate "incomplete" and separated enzyme centres, *e.g.* dehydrogenase and indophenol oxidase plus cytochrome. These are connected by some mechanism for electron conduction in the organised structure of the cell, or by chemical intermediaries. In the latter case the chemical intermediaries during the actual performance of the work compete with oxygen for the metabolite supplying the energy. This conception indicates the manner in which chemical work (syntheses) is performed by anaerobic organisms; and suggests that the main function of oxygen consumption in aerobic organisms (apart from the maintenance of body temperature) is other than in the immediate provision of energy for work. The active phases of muscular contraction and the conduction of the nervous impulse are anaerobic; the participation of oxygen is in the recovery phase. The possible mechanisms adumbrated above whereby energy may be transferred for the performance of physiological chemical work suggest that the place of oxygen is here also, in so far as it participates at all, in a "recovery" phase.

(1) THE EFFECT OF THE ENVIRONMENTAL TEMPERATURE

Before proceeding to the detailed exposition and application of this theory the variation in the specific dynamic action of protein at different temperatures must be considered. Rubner (1902) discovered that at low temperatures, 0–5° C., protein exerts no specific dynamic action. Rubner's data show that the fasting energy production at this temperature is already nearly the resting maximum. Heat developed in any form, whether in oxidative deamination or the surplus energy in the synthesis and excretion of urea, spares carbon from metabolism. We are dealing here primarily with a nervous rather than with a phenomenon of intermediary metabolism.

Even at higher temperatures, 15–20° C., glucose and fat exert no specific dynamic action. The combustion of the products of deamination will accordingly spare an equivalent quantity of endogenous carbon. The heat evolved in the metabolism of the nitrogen, however, appears as excess metabolism, giving a specific dynamic action of 7–8 calories per gram of nitrogen metabolised (see below). The conditions in the frog may be taken to correspond to those of a warm-blooded animal when its chemical heat-regulating mechanism is in operation, *i.e.* at temperatures below 20° C. The values for excess calories over excess nitrogen in dog and frog at this temperature correspond (Table II).

At temperatures above 25° C. where the "chemical" heat-regulating mechanism is inoperative and the body temperature is governed only by the "physical" heat-regulating mechanisms, the specific dynamic action attains its maximum value, because here fat and carbohydrate, and therefore the deaminised residues, now exert some stimulating influence on metabolism.

(2) THE INCREASE IN ENERGY PRODUCTION ATTENDING THE METABOLISM
AND EXCRETION OF THE NITROGEN

The increase in metabolism after the ingestion of protein or amino acids attends and follows deamination; it occurs entirely in the viscera, with the possible exception of a small amount accompanying some glycogen synthesis in the muscles from deaminised residues. The evidence on this point seems conclusive. Katabolism of protein nitrogen in the rat occurs only in the liver and kidney, and mainly in the liver (Borsook and Jeffreys, 1935). Bollman, Mann, and Magath (1926) came to substantially this conclusion earlier from their observations on hepatectomised dogs, though they restricted deamination solely to the liver. This was the conclusion also of Bornstein (1929), and Bornstein and Roese (1929) from perfusion experiments on the isolated organs of the dog. However, Bornstein and Budelmann (1930) found that per unit weight of tissue the kidney formed ammonia from glycine at about the same rate as the liver. The observations of London and his collaborators (1934) suggest that some oxidative deamination may also occur in the intestine. Wilhelmj, Bollman and Mann (1928) found further that after hepatectomy glycine and alanine exert no specific dynamic action. Dock (1931) extended these observations to the rat and demonstrated that the hind-quarters participate very little in the increased oxygen consumption during protein metabolism; and that 80 per cent. of the increase in metabolism can be ascribed to the viscera (liver, kidneys and intestine). There is an increased oxygen consumption when amino acids are added to slices of rat liver (Reinwein, 1928), and kidney (Kisch, 1931). Similarly Bornstein and Roese (1929-30) found an increased oxygen consumption when glycine was added to the blood perfused through the liver. Glycine added to the blood perfusing the extremities was not followed by an increased oxygen consumption, whether the liver was in circuit during the perfusion or not. This result, as mentioned above, stands in contradiction to the positive result obtained by Rapport and Katz (1927). The duration of the experiments of Bornstein and Roese was about half those of Rapport and Katz. Unfortunately Bornstein and Roese do not give the concentration of glycine in the perfusing blood in their experiments. Further, the variations in oxygen consumption in the individual determinations in a single experiment were relatively very large. They cannot therefore be accorded more than qualitative significance. For this reason also their further conclusion that glycine is a protoplasmic stimulant in the liver because the increase in oxygen consumption ran ahead of the ammonia production is not convincing, especially as no data on urea production are given. Nothaas and Never (1930), as stated above, also observed an increased oxygen consumption when glycine was added to the blood perfused through the isolated liver, and none in the extremities. They record no measurements of the rate of deamination, urea or ammonia production.

Though Wilhelmj and Bollman (1928) were of the opinion that the most suitable manner of expressing the relationship between the specific dynamic action and the amino acid administered is as calories per millimol of amino acid deaminised, Wilhelmj and Mann (1930) emphasised that they did not believe nor did they wish

to imply that deamination was always a necessary prelude to specific dynamic action. In view of the evidence against any stimulation of general tissue metabolism, the localisation of deamination, urea formation and the excretion in the viscera, the absence of specific dynamic action in the hepatectomised animal, the observation that most of the specific dynamic action is in the viscera, and the increased oxygen consumption when amino acids are added to the isolated liver and kidney, the burden of proof is now on the thesis that deamination is not a necessary prelude to specific dynamic action.

The peak of the increase in energy metabolism will coincide with the highest rate of metabolism of the protein or amino acids, since the deaminised residues and the nitrogen released in deamination will be metabolised together. This rate will be greater the higher the concentration of amino acids in the tissue fluids (Borsook and Jeffreys, 1935). This is in accord with the observation of Seth and Luck (1925) that the ability of a given amino acid following its ingestion to increase the rate of energy metabolism (this is to be distinguished from the absolute specific dynamic action, *i.e.* the ratio of excess calories to excess nitrogen) parallels its ability to increase the concentration of amino nitrogen in the blood. It is in accord also with the correspondence observed by Csonka (1915) in phlorhizinised dogs between the period of maximum heat production (second and third hours) after the ingestion of meat or glycine, and the period of extra glucose excretion. Once it is excluded that the amino acids act as stimulants of tissue metabolism without themselves necessarily undergoing any metabolism, the conclusion seems unavoidable, from the parallelism between blood amino nitrogen concentration and increase in metabolism, that the stimulus begins with the metabolism of the amino acids. It follows therefore that the magnitude of the stimulus will depend primarily on the rate of metabolism of the amino acid responsible for the increased concentration of amino nitrogen in the blood, and only secondarily on this increased concentration *per se*. The magnitude of the stimulus will also depend on the mode of deamination. For instance, the hydrolysis of arginine, yielding urea and the rather inert amino acid ornithine, will result in a smaller increase in metabolism than the oxidative deamination of alanine to ammonia and pyruvic acid. The nature of the deaminised residue is also significant. Our views regarding the relation between the blood amino nitrogen and the specific dynamic action are in accord with that expressed by Gaebler (1929) at that time in Lusk's laboratory: "Not the amino acid content of the blood, but the avidity with which tissue absorbs amino acid, converts it into urea, and passes this through the blood stream into the urine, parallels the intensity of the specific dynamic action." These considerations are discussed in further detail below.

It would be unjustifiable to conclude categorically that all the excess nitrogen after the ingestion of protein is to be ascribed to the metabolism of the protein fed. Some of the ingested material which has been synthesised into labile nitrogen (Borsook and Keighley, 1935), may be metabolised toward the end of the experimental period, and in so doing may cause to be metabolised with it other nitrogenous substances not ingested with the protein in question, with which it has been com-

bined. But it is highly probable that the extra nitrogen is derived mainly from the ingested material.

The maximum increase in oxygen consumption attending the synthesis of urea from ammonia and carbon dioxide is one molecule of oxygen per molecule of urea, equivalent to about 4 calories per gram of nitrogen (Borsook and Keighley, 1933). This is the maximum possible increase. In varying physiological states the increase may be much less, and may approach zero (Borsook, 1935*a*).

How much of the specific dynamic action can be ascribed to an increased metabolism of the kidney attending the increased excretion of urea is uncertain. Since the last review of this topic (Borsook and Winegarden, 1931*a*) two papers have appeared, one by Dock (1933) who observed in rats for the ratio of work performed in excreting extra urea to increase in metabolism, a value of 20 per cent.—much higher than any previous value—and a second paper by Van Slyke, Rhoads, Hiller, and Alving (1934), who concluded from their observations that increased work of concentration by the dog's kidney entails no increase in metabolism. The data reported in the latter paper hardly warrant the conclusion drawn. The calculated increases in oxygen consumption for the work performed on the basis of an efficiency of only 5 per cent. in two of the three cases fall well within the unaccountable variations in the control and experimental animals. In a personal communication Van Slyke stated: "We... did not mean to state that no energy is used for excreting urea..." From Dock's experiments it seems that our previous estimate of the inefficiency of the kidney in performing work of concentration may have been too low. One of the difficulties in applying values observed for this efficiency in short-period experimental periods to normal physiological conditions is that the efficiency of the kidney increases as its working intensity increases (Dock, 1934). In this connection Drury (1935) made the interesting suggestion that "there might be some mechanism in the kidney—as there is in muscle—for borrowing energy (to be paid up eventually by oxidation, of course, but making the oxygen consumption not a rigid indicator of energy turnover at any particular instant). In other words an oxygen debt mechanism..."

Summing up the increase in metabolism attending the handling of the nitrogen we may assign 4 calories per gram of nitrogen to oxidative deamination, a maximum value of 4 calories but probably less to the synthesis of urea from ammonia, and an undetermined and uncertain quantity, possibly less than 1–2 calories (Dock, 1934) for the excretion of urea. This approximate sum is in good agreement with the minimum values observed for the specific dynamic action in Table II.

Certain special cases, and a possible revision of the estimate of increased metabolism as a result of deamination and urea formation, must be considered. It is obvious, as pointed out above, that urea resulting from the hydrolysis of arginine is accompanied by a smaller heat production than that formed from alanine by way of ammonia. It also entails no additional oxygen consumption. Similarly the liberation of nitrogen from the imidazole ring of histidine (Edlbacher and Neber, 1934) is not an oxidation. As deamination is studied further other examples will doubtless be found. Further, it is possible that in the oxidative

deamination of amino acids in the liver, where most of the deamination probably occurs *in vivo* (Borsook and Jeffreys, 1935), the energy of oxidative deamination (without invoking cyanic acid as a precursor (Werner, 1923), for which no physiological evidence has been found) may be used to convert the nitrogen to urea. Deaminised nitrogen in the liver may not pass through the stage of ammonia. It may be converted directly to urea.

The experiments of Lundsgaard (1931) are in accord with the assignment of a fraction of the specific dynamic action of protein to the increased energy production attending the metabolism of the nitrogen. It is not possible to obtain any quantitative data from his observations because the figures for nitrogen excretion are not given. He found in dogs at about 20° C. that glycine, alanine, glutamic acid, aspartic acid, and tyrosine exerted a specific dynamic action. Over a period of 5 hours there appeared to be an average increase in metabolism for the different amino acids of approximately 8 calories per gram of nitrogen administered. Sodium acetate increased the metabolism, but the increase was less than with an equivalent amount of glycine. Compared with glycine and alanine, glycollic and lactic acids caused only a slight increase in metabolism. Ammonium lactate and ammonium chloride, on the other hand, caused an increase in metabolism of the same order of magnitude as alanine. There are similar earlier observations of Gräfe (1916) that ammonium salts and acetamide exert a large specific dynamic action.

(3) THE INCREASE IN ENERGY PRODUCTION IN THE METABOLISM OF THE CARBON

Even maximum allowance for the metabolism of the nitrogen would leave a large fraction of the normal specific dynamic action of protein (in warm-blooded animals above 20° C.) unaccounted for. Increases in metabolism of 8–10 calories per gram of nitrogen indicate some increased oxidation of carbon. Terroine and Bonnet's estimate (1926, 1929) of 8–10 calories per gram of nitrogen in both cold- and warm-blooded animals is certainly too low for most warm-blooded animals above 25° C. In their view the metabolism of the nitrogen—deamination, urea formation and excretion—is responsible for the whole of the specific dynamic action. The warm-blooded animals they employed (1929) were rabbits. The amounts of amino acids ingested were very large—1.1 gm. alanine and glycine nitrogen per kg. One-third this quantity injected into rats is toxic and there is no specific dynamic action (Lewis and Luck, 1933). It is possible that in the rabbit the deaminised residues spare an equivalent amount of tissue carbon and hence provoke no increase in metabolism. The whole of the specific dynamic action in this event arises, as Terroine and Bonnet proposed, from deamination, urea formation, and excretion. It is interesting in this connection that the concentration of urea in the urine of the rabbit is normally less than in other warm-blooded animals (Mayrs, 1922). The observations of Guttmacher and Weiss (1927) that glycine and glucose have nearly the same specific dynamic action on lightly narcotised rabbits, and that both disappear in deep narcosis also indicate that the control and magnitude of specific dynamic action in the rabbit is not typical. In other animals the specific dynamic action of glycine greatly exceeds that of glucose.

Pointing in the same direction are the observations of Lundsgaard (1931) that the specific dynamic effects of glycine and ammonium salts obtained in narcotised (urethane) rabbits and cats are greatly diminished or abolished after curarisation in the rabbit, but persist unchanged in the cat.

It is immaterial if one considers, with Lusk, that all the ingested carbon is converted to glycogen at the expense of endogenous carbohydrate or fat; or that all the ingested carbon has been metabolised, and some endogenous carbon has been spared. In either case as long as the excess metabolism is below the physiological heat value of the protein or amino acid there is a net sparing (or gain) of tissue carbon.

The metabolism of the carbon is the most variable quota. It is probably for this reason that the total excess metabolism varies with single amino acids to some extent with the amount administered (Lewis and Luck, 1933). Large doses of single amino acids administered *per os* or subcutaneously are toxic and depress the metabolism. We shall consider here only the changes below the toxic level.

The fundamental postulate will be that the products of deamination follow the same course as keto and hydroxy acids of any origin, *e.g.* from carbohydrate in the course of muscular exercise, or in the intermediary metabolism of fat. The deaminised residues may be completely burned, they may be entirely converted to glycogen, or converted to fat. The energy exchange in each of these possible cases will be considered.

When all of the carbon of the deaminised residues is burned, with no sparing of the endogenous carbon, the excess metabolism will be the sum of the physiological heat value of the protein or amino acid plus 7–10 calories per gram of nitrogen. For glycine this will total 18–20 calories, for alanine approximately 30 calories, and for protein 30–35 calories per gram of nitrogen.

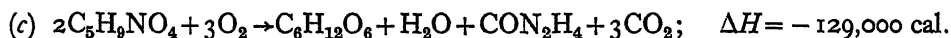
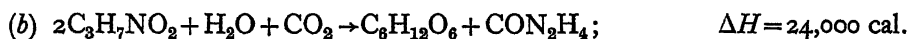
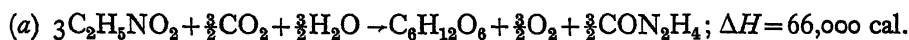
The deaminised residues of glycogenic amino acids may be entirely converted to glycogen—the case considered by Lusk and by Aubel. As a rough approximation we may assume with Aubel, without accepting the details of the coupled reactions which he postulated, that 1 mol of triose is burned for every 4 mols synthesised to glycogen. The increase in metabolism in this process will be 5 calories per gram of nitrogen deaminised, giving a total of 12–15 calories for the ratio of excess calories in excess nitrogen. The minimum and maximum values in Table II are in good agreement with these, as it were, “predicted” values.

It is only rarely that one of the above extreme conditions will prevail. Commonly a mixture of increased metabolism of deaminised residues plus some glyconeogenesis will occur, giving a value at temperatures above 25° C. intermediate between 12 and 35 calories according to the substance burned. The observations of London and his collaborators (1934) suggest how this combination of processes may occur. After the intravenous injection of alanine they found an increase in ammonia and pyruvic acid in the portal blood, indicating deamination in the intestine, with a decrease in pyruvic acid in the blood of the renal vein as compared with the renal artery. Krebs had shown previously (1933*a*) that rapid oxidative deamination of alanine occurs in the kidney, with the formation of ammonia and pyruvic acid. In

order to detect the pyruvic acid in the kidney it was necessary to poison the oxidative mechanism with cyanide. Polonovski and his collaborators (1934) showed that active deamination of alanine occurs in the kidney *in vivo*. Alanine will therefore contribute to glycogen formation in the liver to the extent that the pyruvic acid or lactic acid resulting from its deamination reaches the liver from other sites of deamination, while the pyruvic acid which remains in the kidney is burned.

With glutamic acid and aspartic acid which give rise on deamination to α -keto-glutaric acid and oxalacetic acid (Krebs, 1933*b*) a number of oxidative and other exothermic processes intervene before three of their carbons appear as pyruvic acid. In the case of glutamic acid the probable steps are: ketoglutaric acid, succinic, fumaric, malic, oxalacetic and finally pyruvic acid. Needham (1930) found that glutamic acid was converted to succinic acid in muscle mash. All the other necessary enzymes have been demonstrated. It is to be expected, therefore, from their intermediary metabolism that the specific dynamic action of glutamic acid and aspartic acid per gram of nitrogen deaminised will be greater than that of alanine.

According to Lusk glutamic acid and aspartic acid exert no specific dynamic action (Atkinson and Lusk, 1918; Chambers and Lusk, 1930). This was the fundamental experimental evidence for his theory, that the specific dynamic action of an amino acid represents exclusively the energy expended in converting the deaminised residue to glycogen or glucose. There is no doubt that in the phlorhizinised and even in the normal animal glycine, alanine, and glutamic acid may give rise to some glucose or glycogen. Chambers and Lusk proposed the following reactions:



The first two reactions, as written, are endothermic, the last is exothermic. Chambers and Lusk argued from this that glycine and alanine but not glutamic acid will exert a specific dynamic action. No evidence was adduced apart from the caloric data that the sum of the intermediary processes of these amino acids bore any relation to the reactions postulated. Indeed, even on the basis of their theory, exothermic reaction (c) is the only one where a specific dynamic action seems certain. All other observers disagree with Chambers and Lusk that glutamic acid exerts no specific dynamic action (Gräfe, 1916; Rapport and Beard, 1927; Lundsgaard, 1931; Luck and Lewis, 1934; Stassi, 1934). In Lusk's laboratory Rapport (1924) found that gliadin, which contains about 44 per cent. of glutamic acid, 2.0 per cent. of alanine, no glycine, and only a small amount of the aromatic amino acids (Osborne and Mendel, 1914), exerts a greater specific dynamic action than gelatine which contains 26 per cent. glycine and 9 per cent. of alanine. The probable reason for Lusk's failure to observe any specific dynamic action with glutamic acid is the short period of observation—a few hours—and the slow absorption of this amino acid from the intestine (Johnston and Lewis, 1928). The data of Rapport and Beard suggest that the specific dynamic action of glutamic acid (i.e. the ratio of extra calories to extra

urinary nitrogen) is greater than that of alanine, which is in accord with Rapport's observation on gliadin. Too small a fraction of the nitrogen administered was recovered in this experiment for the absolute value to be relied on. A similar result was obtained by Luck and Lewis. Their data indicate that with non-toxic concentrations the specific dynamic action of glutamic acid is much greater than that of glycine or alanine. This result, as shown above, is to be expected from the intermediary metabolism of glutamic acid.

Aubel and Schaeffer (1932) proposed a detailed scheme of coupled reactions for the conversion of alanine to glycogen and urea. Their scheme for the formation of urea from ammonia and carbon dioxide takes no account of the free energy change, so that in one of their alternatives this reaction is written as proceeding spontaneously. This is impossible, because, under physiological conditions, there is a gain in free energy of 14,300 calories per mol of urea formed. In the coupled reactions which promote this synthesis the stoichiometrical requirements must be satisfied, with the result that a quantity of metabolite will be burned yielding more than the free energy absorbed, since the free energy change ($-\Delta F$) for the whole system must be positive. It is still possible that there may be no increased consumption of oxygen, but there must be oxidation of some metabolite (Borsook, 1935*a*). Furthermore, in the scheme of Aubel and Schaeffer pyruvic acid is depicted as passing to lactic acid by a simple, direct hydrogenation. The work of Embden and his collaborators (1933) and Meyerhof and his collaborators (1933, 1935) indicates that the process is more complicated than this and involves reactions with phosphorylated trioses. On the other hand, the analysis of the specific dynamic action here is in complete accord with their general conception that all reactions which cannot, for thermodynamical reasons, proceed spontaneously must be coupled with other energy-yielding reactions; and especially with their concluding statements:

Il s'ensuit que, toutes les fois qu'une substance sera introduite dans l'organisme pour donner naissance à la suite de réactions à d'autres corps, l'on devra s'attendre à trouver une réaction dynamique spécifique. Les aliments pourraient donc produire une A.D.S. même s'ils n'étaient pas transformés en glucose. On peut même aller plus loin et prévoir que, suivant les conditions l'A.D.S. d'une même aliment peut varier.

It must be added that the necessity for coupled reactions is determined not by the values of ΔH , the change in energy content, but of ΔF , the free energy change. The synthesis of urea cited above is a case in point. Exothermic reactions may also be responsible for specific dynamic action. The largest group of reactions of this type are those where oxygen reacts directly with a metabolite, as, for example, in oxidative deamination. The reactions whereby the deaminised residue of glutamic acid is converted to pyruvic acid, and the oxidation of the benzene ring of phenylalanine and tyrosine also appear to belong to this class. Hence the large specific dynamic action of these substances when expressed in terms of metabolised nitrogen. The smallest specific dynamic action which glutamic acid can exert is 4 calories per gram of nitrogen. This would occur when the reactions whereby α -ketoglutaric acid is converted to pyruvic acid enter into coupled reactions in which all of the energy is used and the pyruvic acid formed spares from combustion an equivalent amount

of tissue glycogen. This, *a priori*, is improbable, and is excluded by the data obtained by all observers except Chambers and Lusk. These data indicate the non-utilisability of the energy released in the formation of pyruvic acid from glutamic acid.

If equations (a) and (b) above represent even approximately the reactions which occur, the specific dynamic action per mol of glycine or per gram of nitrogen metabolised should be twice that of alanine. Actually the value is in most cases the same both in the normal and in the phlorhizinised animal. On this point all observers are agreed. Unfortunately too little is known of the intermediary metabolism of glycine to suggest the answer to this problem. It is possible that the coincidence is more apparent than real. The calculated specific dynamic action for complete combustion of the glycine is approximately 20 calories, and for conversion of all the alanine carbon to glycogen, 12 calories per gram of nitrogen. In view of the probability that the actual metabolism of both these amino acids is commonly a mixture of glycogen formation and combustion the similarity of their specific dynamic effects is not quite so anomalous—especially as a large fraction of the nitrogen administered in these experiments was left unaccounted for at the end of the period of observation.

(4) THE VARIATION IN THE SPECIFIC DYNAMIC ACTION OF PROTEIN IN DIFFERENT NUTRITIONAL STATES

In different nutritional states, according to the prevailing fuel mixture, different values for the specific dynamic action of any one amino acid may be expected when some of the carbon is converted to glycogen or fat. The fuel for this synthesis may be carbohydrate, fat, or even protein. When fat is the main fuel this re-synthesis entails a greater expenditure of energy than when carbohydrate is burned (Borsook and Winegarden, 1930). Hence the specific dynamic action will be greater in a starving than in a well-fed animal (Wilhelmj and Mann, 1930). It must be mentioned here that the abnormally high values for the specific dynamic action of amino acids observed by Wilhelmj and Mann in starved dogs occurred only when 70–85 per cent. of the administered alanine or glycine was not accounted for. In those experiments where larger quantities of metabolised nitrogen appeared in the urine the ratios of excess calories to excess nitrogen were not abnormally high. In spite of the large literature on variations in the specific dynamic action in different nutritional states the only other work in which data were found on the nitrogen excretion is that of McCann (1920). Here a starved subject (man) showed no increase in metabolism and a slightly diminished urinary nitrogen, compared with the basal, during the first 4 hours after the ingestion of meat.

In an animal which has been starved until its stores of carbohydrates and fat are exhausted, glycogen formation from the carbon arising from ingested protein will entail a further breakdown of body protein. Hence the very large quantity of protein required with a diet of protein alone to establish nitrogen equilibrium in an animal which has been starved.

These considerations lead directly to the deductions of Mitchell (1934), that the specific dynamic action of a given protein or amino acid will be less when added to a perfectly balanced diet in which the availability of the metabolisable energy is a maximum than in a poorer diet. As a corollary the specific dynamic action of a given substance is not a constant, but will vary with the nutritional state of the animal, the prevailing fuel mixture, and the nature of diet. It will be a minimum with a perfectly balanced ration.

(5) THE NON-AVAILABILITY OF THE SPECIFIC DYNAMIC ACTION
OF PROTEIN FOR MUSCULAR WORK

The energy liberated in the specific dynamic action of protein will not be available for muscular work. The metabolism of the nitrogen is obviously outside the sphere of the metabolic changes during and after muscular contraction. The deaminised residue in the case of alanine is identical with, and in other cases is analogous to, the products arising in the relaxation and post-contraction phases of muscular contraction. The metabolism after deamination therefore is that of oxidative recovery. Deamination gives rise to an "oxygen debt". The cost of meeting this debt will vary, as discussed above, with the prevailing food mixture. The excess metabolism of muscular work and the specific dynamic action of protein above 25° C. therefore will always be additive. This was observed by Rubner (1910), Anderson and Lusk (1917), and Rapport (1929). Similarly in a diet "the final or net energy value is equal to the metabolisable energy minus the increase in the heat production incident to the consumption and utilisation of the ration. The latter increment consists largely (in farm animals) or entirely (in humans) of the 'specific dynamic effect' of the food" (Mitchell, 1934).

(6) THE SPECIFIC DYNAMIC ACTION OF SOME KETOGENIC AMINO ACIDS

So far the discussion has been concerned mainly with the fate of glycogenic amino acids. An increase in metabolism also follows the administration of some ketogenic amino acids. The greatest increases in metabolism observed per gram of nitrogen metabolised were with ingested phenylalanine (Wilhelmj and Bollman, 1928) and tyrosine (Rapport and Beard, 1927). This again is to be expected from their intermediary metabolism. Oxidative deamination is followed by quinone formation (Medes, 1932), splitting of the ring, the formation of acetoacetic acid, which in turn is either burned or converted to fat. The values observed, 44-56 calories per gram of nitrogen deaminised, large as they are, are less than half the physiological heat values of these amino acids, and indicate incomplete combustion, storage, or sparing of endogenous carbon on the one hand, and on the other that none of the energy in the earlier stages of metabolism of the benzene ring is available for physiological work. Injected tyrosine behaves quite differently (Canzanelli and Rapport, 1933). The increase in metabolism per mol is enormous, suggesting a pharmacodynamic action homologous with di-iodotyrosine and thyroxine.

On some occasions some of the carbon even of the antiketogenic amino acids is probably stored as fat. It occurs with ingested glucose, and there is no reason

to exclude glucose formed from amino acids. In this conversion 11-23 per cent. of the energy of the glucose is lost as heat (Borsook and Winegarden, 1930). We may expect under these conditions a high specific dynamic action. This, possibly, is the reason for those exceptionally high values where the specific dynamic action of protein exceeds by much more than 7-10 calories the physiological heat value of the amino acid.

It is the variation in the metabolism of the carbon which will be responsible for the low values—even when referred to the metabolised nitrogen—of the specific dynamic action of protein in endocrine disturbances.

Lusk (1929) wrote regarding the problem of the specific dynamic action of protein: "The hypotheses which have been presented cannot now be welded into a concordant whole. They transcend one's powers to coordinate them. They are mentioned here so that younger workers may realise the bases from which they can approach a fuller understanding of the cause of the specific dynamic action of protein."

Such a correlation has been attempted here. It is inevitable that so much of the argument should be concerned with the work and ideas of Lusk. He was the first to undertake an experimental analysis of this problem, the first to restate the problem in terms of specific chemical reactions. He, more than any other single worker, kept the problem in the foreground of physiological thinking and experiment for the last 30 years.

V. SUMMARY

When food is ingested by an animal in an environmental temperature above 25° C. his energy metabolism increases. This is known as the specific dynamic action of foodstuffs. The largest specific dynamic action is exerted by protein and amino acids.

The specific dynamic action of injected amino acids, other things being equal, is approximately the same as protein taken by mouth. This and other evidence exclude the work of digestion and absorption as the source of this increase in metabolism. The other of the older hypotheses proposed in explanation of this phenomenon referred the increased heat production and respiration to an increased metabolism of the cells, apart from digestion, absorption and excretion. That of Voit was that the increased metabolism was a plethora effect, the result of an increased concentration of metabolites in the cell; of Rubner that the increased heat production represented reactions in which part of the protein was converted to glucose, the remainder not convertible to glucose is burned with the nitrogen-carrying moiety. The heat produced in these reactions, according to Rubner, is not utilisable by the organism and hence appears only as heat.

Lusk restated these hypotheses in terms of specific chemical reactions and subjected them to experimental test. He first took the position that though the increased metabolism following the ingestion of carbohydrate and fat were plethora effects, the calorogenic effect of amino acids was of different origin and represented a specific stimulation of the cells without the amino acids themselves necessarily

undergoing oxidation. Later he modified this view and held that the specific dynamic action of amino acids represented the heat loss in converting the deaminised residues to glucose, and this specific dynamic effect was an absolute and characteristic constant for each amino acid, in spite of the fact that his observations, as a rule, terminated long before the metabolism of the amino acid administered was complete. Lusk held also that the metabolism of the nitrogen—deamination, urea formation, and excretion—was not responsible for any of the increase in metabolism observed. This contention was based mainly on the observation that glutamic acid exerts no specific dynamic effect, an observation which all other observers have shown to be erroneous. Lusk's explanation fails to account for the large specific dynamic effects of amino acids, such as tyrosine and phenylalanine, which are not converted to glucose.

Although it was emphasised by Lusk (at first at any rate) that the increase in energy metabolism is proportional to the amount of protein or amino acid metabolised, the practice arose, particularly among clinical workers, of considering the increase in energy metabolism observed in the first few hours as an absolute quantity to be referred to the quantity of protein or amino acid administered, rather than to the quantity metabolised in the interval through which the energy metabolism was observed. This is chiefly responsible for the conflicting reports regarding variations in the specific dynamic action of protein in endocrine and nutritional disorders.

The specific dynamic action of protein is not constant. It is usefully expressed as a ratio of calories in excess of the basal to urinary nitrogen in excess of the basal. Nearly all the data on record which can be expressed in this form are collected.

A theory of the specific dynamic action of protein is presented which accounts for the variations, and the minimum and maximum values observed for the ratio of excess calories to excess urinary nitrogen. According to this theory the specific dynamic action is a composite of two factors, one nearly constant, representing the increased energy production attending the metabolism and excretion of the nitrogen, and amounts to 7–10 calories per gram of nitrogen; the other—more variable, and at times larger fraction—arises from the metabolism of the carbon.

Since the amino acids do not act as primary stimulants to cellular metabolism (the evidence for this is discussed in detail) the increase in metabolism follows their deamination—hence the parallel between the increase in energy metabolism and the increased concentration of amino acids in the blood, the increased urinary excretion of uric acid in man, and of glucose in the phlorhizinised dog. For the same reason when the organs in which deamination occurs are removed—the liver, and to a lesser extent the kidneys and small intestine—injected amino acids exert no specific dynamic action; and in the normal animal the specific dynamic action is confined to the viscera.

In the metabolism of the nitrogen, the heat produced in oxidative deamination is not available to the organism for work because the stoichiometrical requirements when oxygen combines directly with a metabolite must be satisfied. For the same reason in the coupled reactions whereby urea and other products (glycogen) are synthesised, the excess energy is not available for physiological work.

The metabolism of the carbon is responsible for the observed variations in the specific dynamic action of protein. In general it may be compared to the recovery phase of muscular exercise. An oxygen debt is incurred, and the cost of its repayment varies with the nutritional state and the nature and fate of the deaminised residue. Accordingly the specific dynamic action of protein is not available for muscular or other work in the organism. It will vary according to the extent that the deaminised residues spare tissue carbon, whether they are converted to glucose or fat, and according to the nature of the fuel mixture supporting these syntheses and conversions.

This theory accounts for most of the hitherto anomalous phenomena in the specific dynamic action of protein. Reference of the increase in metabolism to the quantity of nitrogen metabolised shows that there is no "neutralisation" of the specific dynamic effects of amino acids when these are given with protein; and the specific dynamic action of protein is not particularly low or absent in endocrine or nutritional disorders. Analysis of the physiology of coupled reactions, taking into account the mode of deamination of individual amino acids (whether oxidative or hydrolytic), and the nature of the deaminised residue indicates the reasons for the high specific dynamic effects of ketogenic amino acids such as tyrosine and phenylalanine, and of glutamic acid, and also for certain possible low figures, as in the cases of arginine and histidine.

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THE PASSIVE IRON WIRE MODEL OF PROTO-PLASMIC AND NERVOUS TRANSMISSION AND ITS PHYSIOLOGICAL ANALOGUES

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I. MODELS IN GENERAL

REGARDED from the most general point of view, the use of models—definable broadly, for the purpose of this review, as diagrams, plans, or simulacra of natural phenomena or conditions otherwise difficult to comprehend—is not a special peculiarity of scientific method but is implicit in all attempts at clear mental representation. Thus in the recognition or identification of any ordinary object of experience we compare a mental model, *i.e.* a concept, with the object. In this sense any definite conception of a concrete object is a model; and from simple conceptual models of this type the transition to the consciously designed and often elaborate models or plans used in the arts and sciences is a natural one. No complex task can be undertaken without such models. The model gives concreteness and permanence to the concept and makes possible close examination or even experimentation. Any scientific model of a natural condition or process is a simplification, and as such has the advantage of intelligibility; typically it conforms to the rule of parsimony and reproduces only what are considered to be the essential features of the phenomenon

under consideration. A map is a familiar example; it represents in their true space relations the important permanent features of a locality. The reproduction is relational, not literal. Usually in physical science the model of a complex phenomenon or system aims at representing clearly the spatial and temporal relations of the essential component parts; these components are regarded as having certain fixed properties and modes of behaviour (natural constants); and activity in the system as a whole consists chiefly (or exclusively) in changes in the space-time relationships of the components. Such presuppositions are usual in the mathematical treatment of natural phenomena, as we see in astronomy, mechanics, and many other fields. The natural fact is regarded as agreeing with the model in its relational or structural characteristics only; and for many scientific purposes no further agreement need be implied; the inner nature or intrinsicity of the phenomenon is disregarded. This is merely another way of saying that the model is an abstract representation, not a replica of the natural reality; the agreement with the represented object, while real, is necessarily partial and confined to certain selected features, usually structural.

Biology, because of the complexity of its subject matter and the special need for simplification, has a larger and more diversified assortment of models than other sciences. If we disregard pictures, or other models which aim simply at reproducing the visible aspects of phenomena, we find that in biological models vital processes are usually represented as combinations, more or less complex, of physical or chemical processes. Examples are to be found in any text-book of physiology. Muscular contraction, cell division, glandular secretion, growth, nervous transmission, all have their appropriate models. The assumption is that the processes represented in the models are similar in kind to those of the living system. The agreement need not be complete, but so far as it is real it aids in the understanding of the vital process. It is irrelevant that there always have been mechanistic interpreters, from La Mettrie to the present time, who have gone to extremes in identifying vital phenomena with the purely physical features of models. In so far as the living organism is a physical system, physical methods can and must be applied in its investigation; but it should always be remembered that the abstractions and reconstructions made for purposes of physico-chemical analysis and characterisation give only a partial and in that sense defective representation of the natural reality.

These considerations apply to the passive iron wire model considered in this review, which in certain features of structure and activity resembles closely the irritable and transmissive protoplasmic systems, especially nerve. The nervous system is the chief organ of integration in higher animals; it subserves the requirement—essential for survival—that the various spatially and temporally separated processes of the organism must work in correlation if the system is to retain its normal unity and act as a whole. Integration is the most characteristic feature of vital activity; in animals it is accomplished partly by the transport of chemical substances (the endocrine factor), and partly by the rapid transmission of physical and chemical influence to a distance by a process which is independent of material transfer; by analogy with its chemical counterpart (described below) we may call this process “physiological distance-action”. In the living animal both excitatory

and inhibitory influences are thus transmitted, usually through the conducting protoplasmic tracts called nerves. The resulting changes of physiological activity have necessarily a metabolic basis, and involve variations in the nature or velocity of chemical reactions occurring in the nervous or cellular structures concerned. The general physiological problem, on which the study of the model may throw light, has reference to the nature of the physico-chemical conditions and processes which make such transmission possible. (Cf. the recent discussion on nerve by Hill and others, 1934).

II. GENERAL FEATURES OF NERVE MODELS

In a recent interesting review Ebbecke (1928) has described in some detail the chief models which have been used in the attempt to reach valid physical conceptions of nervous transmission. These include chemical, mechanical, thermal, and electrical types of model, with their various combinations. All suffer from deficiencies of some kind; models which throw light on certain features of excitatory action leave us in the dark on others; this is to be expected when we consider the inevitably partial nature of such comparisons. There is, however, general agreement among investigators as to the special importance of the electrical factors. A universal characteristic of the irritable protoplasmic system is that it is electrically responsive and produces electric currents in its activity. The energy of this activity has its origin in chemical reactions; our attention is therefore directed to the connection between electrical and chemical phenomena. The existence of a close interdependence between chemical and electrical changes is a long-established experimental fact, and now forms one of the main corollaries of the electron theory of matter. We may begin with the question: what is the special role played by electrochemical processes in the irritable living system?

In a classical paper Wilhelm Ostwald (1891) gives various striking instances of the phenomenon which he calls "chemical distance-action" (*chemische Fernwirkung*). The term has reference to the influence which the chemical reactions occurring at the surface of one electrode in a battery or electrolytic cell have upon those at the other electrode. If an oxidisable compound (*e.g.* a ferrous salt) is in contact with one of two platinum electrodes (*A*) connected by a wire and dipping into an electrolyte solution, and an oxidising reagent (*e.g.* HNO_3 , bromine, bichromate, etc.) is placed in contact with the other electrode (*B*)—no matter at what distance from the first (provided the electrical resistance of the circuit is not too high)—the compound at electrode *A* is instantly oxidised, while that at *B* is reduced. A galvanometer in the circuit shows that an electric current (positive stream) is flowing in the wire from *B* to *A*. Without a complete circuit and a flow of current there is no reaction. The phenomenon is a manifestation of the dependence of chemical change on electrical change; for every molecule (or equivalent) oxidised at the anode (*A*), a corresponding one is reduced at the cathode (*B*). This characteristic rule, the general law of electrochemical polarity, is exemplified in all cases of electrolysis. In chemical distance-action there is no necessary direct contact of oxidising and reducing compounds, and no transfer of material in the usual sense.

For every electron lost to the anode one is gained at the cathode; but the electrons need not be identical (since all electrons are substitutable for one another); all that is required is that the loss from *A* should be balanced by a corresponding gain at *B*; molecules losing electrons are oxidised, those gaining electrons are reduced; the transmission of chemical influence is automatic and instantaneous so long as the current is free to flow.

This electrochemical polarity at once recalls to the physiologist the physiological polarity of irritable tissues as illustrated, for example, in the law of polar stimulation: excitation occurs at the cathode, depression at the anode, when a current is passed through a nerve or a muscle. When we remember that a nerve may transmit impulses at a rate of 100 metres per second or more, and that the transmission is always associated with a local electric current which develops with extreme rapidity (rising to its maximum in 0.3 σ or less in some motor nerves), the hypothesis naturally suggests itself that some form of chemical distance-action is the basis of protoplasmic transmission. In no other way does it seem possible to account for the observed combination of polar action, metabolic change, electrical change and high velocity of transmission.

Chemical distance-action is illustrated by the following simple experiment. A straight length of platinum wire is placed in a bath of dilute H_2SO_4 ; no effect is seen. If then we touch the wire at one end with zinc, bubbles of hydrogen instantly start out from the wire, not only at the contact but along its whole length, although diminishing with distance from the contact. A gradient of action is thus seen; the chemical effect falls off with distance and becomes inappreciable beyond a certain distance if the wire is long enough. This gradation recalls certain physiological gradations, such as the decrease of dominance or morphogenetic influence with distance from a rapidly growing area, as shown in the work of Child and others. Another experiment of a related type suggests the physiological analogy still more strongly (Lillie, 1917). A small strip of zinc (*e.g.* 50 by 3 mm.) is attached at one end to an iron wire of the same length and placed in a 4 per cent. solution of potassium ferricyanide. Within a few minutes a precipitate consisting of thin-walled vesicles and tubules of zinc ferricyanide appears upon the surface of the zinc, most abundantly near the contact with the iron and decreasing gradually with distance. The iron wire remains bright and bare and shows at first no reaction with the K_3FeCy_6 , even at a distance of some centimetres from the contact. If, however, the wire is separated from the zinc, *e.g.* by cutting with scissors, immediately dark green filaments and tubules of iron ferricyanide begin to form from its surface which within a few minutes becomes covered with a characteristic felt-like growth. The zinc after separation from the iron ceases (or almost ceases) to form precipitation structures. Two features of this experiment are to be noted; first, the zinc, in the absence of contact with the iron (or other metal more positive than zinc), reacts very slowly with the solution, while the iron reacts quite rapidly without contact. Second, contact between the two metals accelerates the formation of precipitation structures from one, the zinc, and represses their formation from the other; *i.e.* there is a reciprocal influence which is exercised through some distance, decreasing as the

distance from the contact increases. We thus see that distance-action may manifest itself either in the promotion or the repression of chemical reactions at the electrode surfaces; and analogous physiological distance-effects (acceleration, reciprocal inhibition) are well known. In the inorganic system the explanation is simple. A miniature electric couple, in which the zinc is anode and the iron cathode, is formed when the two metals are in contact and immersed in the electrolyte solution; the zinc ions as they pass into solution form the precipitate of zinc ferricyanide, while at the iron, where the positive stream is passing from solution to metal, the separation of iron ions is prevented; hence no precipitate is formed until the contact is broken. The characteristic reciprocity depends on the flow of electric current, which determines the electrochemical effect with the associated distance-action.

The possibility that transmission of physiological influence in nerve might be an essentially electrochemical phenomenon was early recognised by students of electrophysiology. Du Bois-Reymond referred the stimulating action of the electric current in irritable tissues to an electrolysis; and Hermann (1879) pointed out that the direction of the local electric currents flowing between the active and inactive areas of a stimulated nerve was such that excitation of resting regions and inhibition of active regions would be an automatic consequence, provided the currents were sufficiently intense. He attributed the spread of excitation to the polarising action of such currents (Strömchen hypothesis). Electrical stimulation, according to his view, was primarily an effect of polarisation; and he attached special importance to the high electrical polarisability of living tissues, which is comparable to that of indifferent metallic electrodes (like platinum) immersed in salt solutions. Hermann (1872-3) also made a special experimental study of the phenomena of polarisation in artificial models in which the polarisable metal and the electrolyte solution were arranged in a manner suggested by the structure of nerve (*Kernleiter* or core-conductor models). In such a model a wire (*e.g.* platinum) passes axially from end to end of a glass tube containing an electrolyte solution, *e.g.* of ZnSO_4 , and provided at intervals with side tubes by which electric connection may be made (in this case by zinc electrodes) with the solution. When a current from a battery is passed through the solution, the wire can be shown (by galvanometers connected with the side tubes) to be electrically polarised for a considerable distance on either side of the battery leads. The distribution of the polarisation currents in the regions beyond the electrodes resembles closely that of the extrapolar electrotonic currents observed in a nerve polarised by a constant current (for fuller details on polarisation cf. Ebbecke, 1933). So far as the purely electrical phenomena of polarisation are concerned the model reproduces the conditions found in the living system, but the resemblance ceases there. No automatic propagation is seen; the polarisation falls off exponentially with distance, and shows nothing of the self-renewing property characteristic of the action currents of a nerve. Something further is required to make the resemblance more nearly complete. This further requirement would seem to be a connection between changes of polarisation at the surface and the occurrence there of definite chemical reactions giving rise to local electric currents with resulting changes of polarisation at regions beyond. We should expect that a system having these

properties would exhibit the property of automatic self-renewal of the local polarisation currents and transmission to an indefinite distance, as observed in nerve. (Recent important experimental and theoretical studies on core-conductor models, made with polarisable collodion membranes, are those of Labes and Lullies, 1932.)

III. SPECIAL FEATURES OF PASSIVE IRON MODEL

In the experiment with platinum wire in acid, cited above, the chemical effect, formation of hydrogen, is an index of the passage of an electric current between metal and solution, with associated electrolysis. An electric couple is formed by the zinc and the platinum, yielding a current which has polarising and chemical action. Hydrogen ions are deionised at the platinum surface (the cathode of the couple) and form molecular hydrogen which appears as bubbles for some distance beyond the contact with the zinc. In this case there is a definite chemical effect, resulting from the change of polarisation, but the effect is strictly local and shows no propagation beyond a limited distance. We can, however, obtain a system capable of unlimited propagation if we substitute for the platinum wire an iron wire and use instead of



Fig. 1. Indicating the conditions of the local circuit at the boundary between the active and the passive areas of an iron wire in nitric acid, the direction of the current (positive stream) is indicated by the arrows, the active region (shaded) being anodal, the passive cathodal. The local intensity of the current in the passive region (and hence the reducing or activating effectiveness) decreases in the order $A < B < C$; beyond a certain distance from the boundary, e.g. XY , it will be insufficient to activate.

H_2SO_4 the strongly oxidising HNO_3 . In this manner we obtain the system, passive iron in nitric acid, the model under consideration in this review. This system is found to resemble in many details of its behaviour a typical irritable and transmissive living system such as nerve (Lillie, 1918, 1920, 1922). It should be noted that the iron model agrees with a core-conductor system in its general structural arrangement; and its properties depend similarly on variations of polarisation at the interface between the two conducting phases. The characteristic difference of behaviour depends entirely upon the presence of a thin reactive layer or surface film, consisting of a higher iron oxide, which is deposited at the surface of the metal under the oxidising influence of the HNO_3 . This layer, so long as it is intact, is highly resistant and impermeable to the acid, and hence prevents the latter from reacting with the iron. The iron thus protected by the oxide film is non-reactive or "passive". The film, however, is readily broken down or destroyed by mechanical, electrical, or chemical influence; and wherever this occurs, exposing the iron underneath, a change of potential follows, the altered region becoming anodal (more negative) to the extent of ca. 0.7 volt. Hence a strong local current arises between the altered region of the wire and the unaltered regions beyond, where the film is still intact; these regions are cathodal, and wherever the local current has sufficient intensity or

density (intensity per unit area of surface), the film is reduced and broken down. Since the same effect is repeated at the boundary between this secondarily altered region and the adjacent unaltered region, the state of activity is automatically propagated over the whole length of the wire (see Fig. 1).

The characteristic behaviour of the system thus depends on the presence of a thin interfacial layer which has different chemical and physical properties in the oxidised and in the reduced states. In the oxidised state it forms an impermeable film which limits or arrests chemical interaction between the phases; when this film is reduced, it undergoes a change of structure which destroys its impermeability and allows the iron to react with the acid; this effect spreads rapidly as just described. A further remarkable feature in the behaviour of the system should be noted at this point; each area of iron, as soon as it becomes active, becomes at the same time anodal, and hence is subjected to the anodal oxidising influence which tends to reoxidise the iron. In strong HNO_3 the film is thus automatically reformed almost as soon as it is broken down, so that the local reaction has a self-limiting or temporary character.

I shall not attempt in this review a full description of the properties and behaviour of the passive iron wire. The essential phenomena are easily demonstrated; if a straight steel wire (*e.g.* piano wire) is dropped into a trough of HNO_3 of, for example, 60–70 v. per cent. concentration,¹ there follows immediately a brief effervescence with darkening of the metallic surface, lasting for 1 or 2 sec., after which no further reaction is shown. The iron is then in the so-called passive state; but if, after an interval, it is touched locally with zinc or ordinary iron, or scraped with glass, a striking effect is seen; instantly a reaction like that just described, associated with effervescence and darkening, flashes from one end of the wire to the other. In acid of this concentration, the reaction is momentary, and the wire reverts automatically to the passive state. This state is one of stable equilibrium—like the resting state in a nerve—from which the system is aroused into activity only by some external change analogous to a stimulus. This inciting or activating change may be electrical, mechanical, chemical (*e.g.* contact with a reducing substance) or thermal. All that is necessary is that it should alter the film to a sufficient degree. It is important to note that the passive state is stable only in acid of more than a certain concentration (approximately 50–53 v. per cent.); in weaker acid the reaction, once initiated, continues actively until the whole wire is dissolved. This persistence of activity in weak acid has a special interest in relation to certain other quasi-physiological properties of passive iron, especially its tendency to rhythmical reaction under certain conditions (*cf.* below, p. 197).

The condition determining passivity is the presence of a thin film of higher oxide adhering closely to the metal surface (Evans, 1927; Freundlich *et al.*, 1927; Hedges, 1928). This film is impermeable to the acid, and its electromotor properties are similar to those of a noble metal. Both these characteristics are important; the impermeability because it prevents or limits the reaction with the acid, and the electro-positive or cathodal character because it is the condition for the formation of local

¹ Volumes of HNO_3 , C.P., of specific gravity 1.42, in 100 volumes of solution.

currents having the qualities of intensity and direction required to reduce the oxide film at a distance beyond the already active area. The rapid spread of activity is an effect of cathodal reduction; this occurs through a certain distance beyond the active-passive boundary, and is associated with disruption of the film. Any local alteration which interrupts the film and exposes the iron underneath gives rise, if sufficient in extent, to a local circuit and initiates an activation wave.

Because of the automatic tendency of local active states to spread, a passive wire reacts in an "all or none" manner. The reaction may be compared to a two-dimensional explosion, with the difference that the spread of activity depends not on local rise of temperature and pressure (as in most types of explosion), but upon the electrolysis produced by local electric currents. Another remarkable peculiarity of the wire, shown in acid of more than a certain concentration (*ca.* 53 v. per cent.), is the property of automatic repassivation mentioned above; a fresh reactive layer or passivating oxide film is deposited automatically on each reacting area of surface as soon as the reaction has proceeded to a certain point. The progress of the local reaction is opposed by the accumulation of insoluble higher oxide; and if this process is rapid enough it soon brings the reaction to rest; *i.e.* the reaction is limited by conditions to which it itself gives rise and which act to restore the original conditions. Evidently a system whose activity depends on an energy-yielding chemical reaction occurring in a thin surface layer would be quickly exhausted, *i.e.* would lose the power of further reaction, unless the reactive layer were renewed. In the passive iron system the destruction of the film furnishes automatically the conditions for its renewal, and in this respect also there is a parallel to the conditions in an irritable living system like nerve, in which automatic recovery or repair follows each excitation (compare Claude Bernard, 1879, p. 127).

IV. ELECTRICAL ACTIVATION OF PASSIVE IRON

It will be apparent from the foregoing that activation of the passive wire, like stimulation of an irritable tissue, is primarily an electric phenomenon; and the conditions of electrical activation show in fact a close quantitative agreement with those of electrical stimulation. What we may call the law of polar activation in the wire is evidently a manifestation of the general law of electrolysis; reduction, on which activation depends, is a cathodal, oxidation an anodal phenomenon. The main conditions of electrical activation may be demonstrated by supporting two iron wires side by side in a vessel containing, for example, 70 v. per cent. HNO_3 , and connecting them through a pole-changer and rheostat (for varying the potential) to a set of storage cells. The following points of agreement with irritable tissues can then readily be shown: (1) polar activation; (2) threshold phenomena, *i.e.* absence of activation below a certain intensity of current; (3) importance of current density (*i.e.* current traversing unit area of wire); (4) activation by rapidly but not by slowly changing currents; (5) changes in susceptibility to activation during the flow of a constant current, *i.e.* while a polarising current (of subthreshold intensity) is flowing between the two wires, the anodal wire becomes more resistant to mechanical or

electrical activation and the cathodal wire less resistant (analogy to electrotonus in living tissues); (6) summation phenomena, *i.e.* activation by a rapid succession of mechanical or electrical stimuli, ineffective when applied singly; and finally (7) activation by the break of an already flowing constant current. This last demonstration requires some modification of the experimental conditions, since the polarisation current, which flows in the reverse direction (through the rheostat wire in the arrangement just described) when the battery circuit is broken, is too weak to activate the wire in 70 v. per cent. acid. But if dilute acid is used (20–25 v. per cent.) in which a passive wire is highly susceptible to activation, and a platinum electrode is substituted for one wire as the cathode of the polarising current, the other wire (the anode) is instantly activated when the current is broken. In order to secure this effect the resistance in the polarisation current circuit must not be too high, and the polarising potential must be sufficient. "Break activation" in the model, as apparently also in the living system, is a case of activation by the polarisation current (for fuller details on electrical activation cf. Lillie, 1935).

Since Hermann's time it has been recognised that electrical stimulation depends on a change of polarisation. The polarisation caused by the stimulating current must have a definite orientation (cathodal, not anodal) and must be reached with a certain rapidity (*i.e.* rapidly changing currents stimulate, while slowly changing ones do not) and maintained for a sufficient length of time. The quantitative relation between the intensity and the minimal duration of a stimulating current has been investigated by many physiologists, and many formulae have been proposed to describe this relationship (for reviews cf. Cremer, 1929; Lapicque, 1926; Monnier, 1934). In general, as the strength of current increases above the threshold or rheobasic level, its necessary duration decreases. Nernst's formula ($i\sqrt{t} = \text{constant}$) is very generally but not universally applicable; this relationship was deduced by Nernst (1908) from the theoretical consideration of the polarisation produced by a current upon a membrane partitioning a solution and difficultly permeable to ions. The tendency of the ions to diffuse away from the membrane—increasing in proportion to their accumulation at its two surfaces under the influence of the current—opposes the polarisation and makes its rate of development less than proportional to the duration of flow; and the square root relation holds approximately through a considerable range of intensities. The charging of a condenser by a current, especially an imperfectly insulated or "leaky" condenser, follows rules of a somewhat similar kind (cf. Ebbecke, 1926–7; Bishop, 1928*a*; Hill, 1932); and there is now a general agreement that the change of polarisation caused by the stimulating current at the semi-permeable membranes bounding the irritable elements is the primary physical event in electrical stimulation. After a certain critical change of polarisation has been thus produced, the living system responds with its characteristic type of behaviour (muscular contraction, initiation of nerve-impulse, etc.). The physiological event or response is initiated or released by the physical change of polarization; but the event itself has its own characteristics which are determined by the special organisation and inter-connections of the irritable system.

The phenomena of electrical activation in the passive iron wire are consistent

with this general conception, and the intensity-duration relation is similar to that observed in living tissues, as the following example shows (Table I). In this experiment the potential of the activating current was varied by a rheostat and the minimal durations required to activate the wire were determined with the Lucas pendulum. The curve relating the intensities and durations resembles closely that of a typical irritable tissue; the values of "rheobase" and "chronaxie" correspond closely to those of a somewhat slowly responding tissue such as vertebrate heart muscle.

Table I. *The wires used were pure low carbon (<0.1 per cent. C.) soft iron ("Armco"), 2 mm. in diameter, with 6 cm. lengths exposed to the acid, 70 v. per cent. HNO₃ at 21°; the battery consisted of six storage cells connected to the tube rheostat through a key and pole-changer. A Lucas pendulum (vibrating spring interrupter) was used to obtain brief currents of known durations. The recovery interval (time elapsed since previous activation of the same wire) was 1 min. in every case.*

E.M.F. of activating current (% of rheostat potential) (t)	Least effective duration of current (σ)		Nernst product ($t\sqrt{i}$)	
	Wire A (t)	Wire B (t)	Wire A	Wire B
90	7.0	7.5	238	243
80	9.2	9.5	244	248
70	11.3	12.0	235	242
60	12.7	15.1	213	234
50	16.6	19.0	205	218
40	22.8	27.0	190	208
30	30.1	33.3	165	173
25	40.8	53.4	160	183
20	ca. 92	ca. 92	192	192

Too brief a current will not activate the wire, because the polarisation produced is insufficient. When polarisation reaches a certain stage, corresponding to the decomposition potential of the system, reduction occurs at the cathode and activation results. We infer that the total duration of the activating current is made up of two periods: (1) the time required to reach the critical level of polarisation, or polarisation time, and (2) the time required to reduce the film sufficiently for activation, or activation time. Polarisation time and activation time are also distinguishable in living tissues; and in nerve it has recently been shown with the oscillograph that an electrical effect referable to physical polarisation can be distinguished from an immediately succeeding bio-electric variation characteristic of the active response of the tissue (Bishop, 1928; Schmitz and Schaefer, 1933; Schaefer, 1934).

V. NATURE OF ACTIVATION PROCESS

The establishment of a certain degree of polarisation (*i.e.* P.D. between metal and solution), under conditions in which a flow of current across the interface is possible, is the essential condition for electrical activation, as in any other case of electrolysis. This critical potential is the decomposition potential, which varies characteristically

in different cases of electrolysis. After the necessary level of polarisation has been reached, electrolysis begins, and, according to Faraday's law of electrochemical equivalence, the number of molecules oxidised or reduced at the electrodes is proportional to the quantity of current passing (*i.e.* 96,500 coulombs per gram-equivalent). In the living system also it is clear that some chemical or metabolic effect is produced by the current and that the physiological response follows directly or indirectly upon this; and many facts, especially the general association of increased permeability with excitation (for the evidence of this cf. Bayliss, 1927; Höber, 1926; Lillie, 1923), indicate that the primary seat of this reaction is the semi-permeable surface layer or plasma membrane. There are difficulties, however, in regarding the cell surface as equivalent to an electrode surface, since conductors of the first class are apparently not present in the living system. It is usual to assume that in any circuit in which there is direct interdependence between chemical action and flow of current, a combination of electronic (or metallic) and electrolytic conductors must be present. Electrons pass between the electrolytic and the electronic conductors (or what corresponds) and this transfer is associated with oxidation at one electrode area and reduction at the other. We need not regard it as impossible that electronic conductors are present in the plasma membrane (*e.g.* carbon chains set side by side might conceivably so act), but we cannot at present identify any of the known components of the membrane with such conductors. It is, however, well known that under certain conditions chemical effects are produced at the surface of a chemically indifferent membrane partitioning an electrolyte solution through which a current is passed; this is the phenomenon called electrostenolysis (cf. Freundlich, 1927). The conditions are that the fall of potential across the membrane should be steep, *i.e.* that the membrane should be thin and have high resistance, and that the current should flow for a sufficient time. Under these conditions, if readily oxidisable compounds are present in the solution, oxidation occurs at the face of the membrane opposite the cathode, *i.e.* where the positive stream passes from membrane to solution, and reduction at the opposite face (Lillie and Pond, 1922). Recently E. S. Fetcher (1934) of the University of Chicago has shown that in the oxidation of ferrous to ferric ions by electrostenolysis at membranes of cellulose acetate the ratio of chemical change to the quantity of current passing the circuit does not obey Faraday's law, but that many faradays (in his experiments usually some hundred) must pass for each equivalent oxidised or reduced. The reason why this law does not hold for electrochemical decomposition at membranes is apparently because a large part of the current can pass between membrane and solution without separation of free electrons (in contrast to the case at a metallic electrode) by simple transport of ions through the pores of the membrane. If, however, the fall of potential across the membrane is sufficiently steep—*i.e.* if the membrane is thin and the P.D. between its two faces considerable—a certain proportion of dissolved molecules, varying according to the conditions of the experiment, are oxidised or reduced when the current is passed. There is also evidence from Fetcher's experiments that the presence of catalysing agents can increase this proportion; thus the electrostenolytic oxidation of methylene blue is facilitated by the presence

of small quantities of iron salts. Evidence thus exists, from the purely physical as well as from the physiological side, that a membrane may be the seat of electrochemical change when traversed by an electric current.

A membrane surface is thus comparable to an electrode surface, with certain reservations. Its electromotor behaviour under certain conditions is closely similar to that of a metallic electrode, *e.g.* with respect to the logarithmic relation of the membrane potentials observed in living organisms to the concentration of ions in the solution (Loeb and Beutner, 1912); a similar condition is shown by certain types of artificial membrane (Michaelis, 1925-6) as well as by interfaces between electrolyte solutions and non-aqueous phases containing weak acids (Beutner, 1920). The "membrane theories" of electrophysiology refer the bio-electric potentials and their variations to the properties of the semi-permeable membranes enclosing the living protoplasm (for membrane theories cf. Höber, 1926, chap. XII). Considerations of this kind justify the conclusion that in the living irritable system (*e.g.* nerve) as well as in the inorganic model local variations of polarisation, when they occur, will give rise to local circuits associated with electrochemical reactions, and that local activity of this kind may be transmitted over the surface under conditions of essentially the same nature as those present in the passive iron wire. In the living system the condition for such transmission is the presence of a thin polarisable and chemically reactive layer at the boundary between protoplasm and external medium.

VI. CONDITIONS INFLUENCING RESPONSIVENESS

In the iron wire as well as in the living tissue, the responsiveness to electrical activation varies greatly under different conditions. It also varies, under conditions otherwise constant, with wires of different composition, being in general greater with the relatively carbon-free soft iron than with steel. With any particular kind of wire these variations of responsiveness can best be measured by determining under different conditions the least durations of current required to activate the wire with currents of known intensity (see Table I). The susceptibility to electrical activation is found to decrease rapidly as the concentration of HNO_3 increases; the threshold of activation is also raised by the presence of surface-active compounds; it also varies characteristically with the interval elapsed since a previous activation (Lillie, 1935). To illustrate: in a typical series of experiments with HNO_3 of 80, 70 and 60 v. per cent., using a current of constant E.M.F., the minimal durations for activation were as follows: in 80 v. per cent., 6.3σ ; in 70 v. per cent., 3.1σ ; in 60 v. per cent., 2.0σ . In a wire activated at different intervals after a previous activation, the critical durations of current were as follows: after 20 sec. of recovery, 39σ ; after 30 sec., 25.2σ ; after 60 sec., 14.8σ ; after 2 min., 12.7σ . Responsiveness thus increases rapidly at first, and then more slowly, finally reaching a constant level; the time curve is similar to the recovery curve of a nerve or a muscle during the relative refractory period. Experiments on the activation of a wire in 70 v. per cent. HNO_3 containing 1 v. per cent. amyl alcohol gave a minimal current duration of 21.2σ , as contrasted with 7.2σ in the pure acid; correspondingly the rate of transmission of an activation wave

was reduced in about the same proportion. This retarding influence of surface-active compounds on activation and transmission has its biological analogue in narcosis in living tissues; in both cases it appears to depend on an interference of the adsorbed molecules with the surface reaction. So far, however, I have not succeeded in finding conditions under which the passage of an activation wave in an iron wire is blocked completely by the presence of a surface-active compound.

These variations in the responsiveness of the iron wire to electrical activation have their close parallels in the behaviour of irritable living tissues. A good illustration is the manner in which the electrical sensitivity of frogs' voluntary muscle varies with the concentration of calcium ions in the medium (Chao, 1935). As the CaCl_2 in Ringer's solution is increased by degrees (*e.g.* through the range from 0.5 to 4.0 millimols per litre), the threshold intensity of the stimulating current shows a regular parallel increase. This effect is to be correlated with the general stabilising influence of the Ca ion, which renders alteration more difficult and favours recovery from an altered state; this influence is also shown in the shortening of the refractory period under the influence of calcium (Bazett, 1908; Graham, 1933). Similarly, in the iron wire, increasing the concentration of HNO_3 increases the stabilising or passivating influence, so that a greater intensity of current is required to reduce the film against the opposition of this influence. An interesting analogy is seen here with the conditions assumed by E. F. Blair (1932) in his recent mathematical study of electrical excitation. He pictures the excitation process as occurring in accordance with the general conditions represented by the equation, $\frac{dp}{dt} = KV - kp$. This states

that the development of the state of excitation (p) in time (t) increases in rate with the voltage (V) of the stimulating current and is automatically opposed by a counter-process which at any time is proportional to the state of excitation then reached. In the iron wire, the nature of the counter-process opposing activation is definitely known; it consists partly in the oxidative action of the HNO_3 , which automatically becomes greater as the altered area of film is increased, and partly in the anodal passivating influence acting at the altered area. Blair's formula after integration

takes the form $\log \frac{V}{V-R} = Kt + C$, in which V is the applied voltage, R the rheobasic voltage, t the duration of the current, and K and C constant. The passive iron wire also conforms to this rule (Lillie, 1935), in common with a large number of irritable living tissues.

VII. SPECIAL FEATURES OF TRANSMISSION PROCESS

The simplest biological comparison of the passive iron wire is with a non-medullated nerve axone. In both systems an internal phase (iron, protoplasm) is separated by a thin, chemically alterable film (oxide film, plasma membrane) from an external phase which is an electrolyte solution containing oxidizing compounds (HNO_3 , oxygen). Although the two systems differ as widely as possible in their special chemical composition, their general structural resemblance carries with it certain definite correspondences in properties and behaviour. The important

common feature, in virtue of which the inorganic system acts as a model for the nerve, is that locally induced alterations in the structure or permeability of the film give rise to corresponding variations of potential, with the production of local currents; these, if their intensity and duration are sufficient, produce secondarily chemical and structural alterations in the film at a distance beyond the altered area, with consequent repetition of effect and transmission.

Transmission in nitric acid of more than a certain concentration (*ca.* 55 v. per cent.) has a wave-like character; *i.e.* an active area of limited length moves continuously over the surface of the wire. This characteristic feature is a consequence of the automatically self-limiting character of the local reaction; this passes through a definite cycle: an area of the passivating film is first broken down by reduction, and then a new film is formed by reoxidation. When the metal beneath the film is exposed by the local breakdown, it immediately reacts with the HNO_3 ; at the same time it becomes the anode of the local circuit formed by the altered area with the unaltered area beyond as cathode; hence it is subjected to the anodal oxidising influence in addition to the direct oxidising influence of the acid. These two actions combine to reoxidise the metal and restore the film. Accordingly the local reaction lasts only until the film is restored; the rate of this restoration (*i.e.* repassivation) increases rapidly with the concentration of HNO_3 , and apparently also with the local density of the current of the active-passive circuit. It seems probable that in the nerve impulse also the re-entrant action current similarly plays a part in recovery; this is indicated by the recent observations of Blair and Erlanger (1933), showing that a subthreshold induction shock delivered during the refractory phase hastens the return to normal at its anode, while it delays recovery at its cathode. (Cf. also Erlanger and Blair, 1931.)

As already described, the local reaction of the iron in strong acid is temporary; and in such a case the length of the simultaneously active region (or activation wave) of a transmitting wire is limited, becoming shorter as the concentration of acid increases. The length (l) of this active area is determined by the product of the local duration (t) of the reaction into the speed (v) with which the reaction spreads ($l=vt$); it varies greatly with the conditions (of concentration, temperature, kind of wire, electrical conductivity, presence of foreign substances, etc.) determining the local duration of reaction and the speed of travel. The characteristics of the activation wave are most readily observed when the wire is enclosed in a glass tube filled with HNO_3 ; the electrical conductivity of the column of electrolyte is then proportional to its sectional area, and the distance through which the local current is effective in reducing the film varies correspondingly, becoming shorter as the calibre of the tube decreases. In a narrow tube (*e.g.* 3–4 mm. in diameter) a steel wire in 70 v. per cent. HNO_3 , activated by touching with zinc at one end or scraping with glass, shows a dark effervescent active area 2–4 cm. long travelling along the tube at a rate of several centimetres per second; this area is bounded on either side by inactive areas. A trail or wake of bubbles is left behind the wave, and after its passage the wire remains dark for some time. It gradually becomes brighter, at the same time as it recovers its former properties.

The speed of transmission of the activation wave varies greatly according to conditions, from many metres per second in a wire lying free in a large volume of acid, to a few centimetres per second in a wire enclosed in a tube. It should be noted, however, that the speed with which states of activity may be transmitted from region to region is not limited by the possible speed of continuously travelling waves, but may be much greater under certain conditions, as in the case of distance-action between discontinuous electrode areas (see below, p. 199). In the case of activation waves under the conditions so far described the speed of travel depends on a number of factors (cf. Lillie, 1925, 1929*a*, 1935), including the temperature (Q_{10} of 1.5–2.5), the presence of surface-active compounds (alcohols, esters, chloroform, etc.), and the electrical conductivity of the medium; it is also dependent on the interval elapsed since a previous activation (analogy with the refractory period or fatigue). These variations of speed are to be referred chiefly to variations in the rate at which the passivating film is reduced under the special conditions of the experiment. Some of the more important biological analogies are discussed below.

In wires enclosed in tubes, the velocity of transmission is found to be nearly proportional not to the sectional area (corresponding to the conductivity of the column of acid), but to the *square root* of the area, *i.e.* to the diameter of the column (Lillie, 1925). This relation is interesting, since it seems to correspond to the conditions found in nerve, where also a nearly linear relation between velocity and diameter has been observed by a number of investigators (Lapicque and Legendre, 1913; Gasser and Erlanger, 1927). The theoretical explanation of this correspondence is not quite clear; but the area of the active region (equivalent to one of the electrode areas), as well as the conductivity of the local circuit, appears to be a factor. In the case of nerve it has been pointed out that if the area of the active region is proportional to the diameter (D) of the axone (*i.e.* $= K_1 D$) and the electrical conductivity of the local circuit—and hence the current intensity—is proportional to the sectional area (*i.e.* $= K_2 D^2$), the current density at a point in advance of the active area should theoretically be proportional to the diameter, *i.e.* to intensity divided by area ($= K_3 D$). Since the secondary stimulating effect of the local action current depends on its density, the speed should vary with the diameter (Gasser and Erlanger, 1927). But this reasoning assumes constancy in the E.M.F. between the active and resting areas of all nerves—which is not certain—and it is further doubtful if a direct proportionality between diameter and active area can be assumed. Blair and Erlanger (1933) have recently published evidence indicating that action potentials increase linearly with speed of propagation, and if this is true the speed should vary with the square rather than with the first power of the diameter. The question is open; in particular the smaller fibres seem to deviate widely from the simple linear rule; the duration of the bio-electric variation may also be a factor (cf. Douglass, Davenport, Heinbecker and Bishop, 1934).

If transmission in nerve and other irritable tissues is an effect of secondary stimulation by the current of the local active-resting circuit (as the local action theory of transmission holds), its speed (s) should be proportional to the rate of development (r) of the local bio-electric variation, and also to the maximal distance

(l) beyond the active-resting boundary at which the current is effective as stimulus; *i.e.* $s = K/l$, where K is a proportionality factor. The rate (r) may be taken as the reciprocal of the time occupied by the rise of the electric variation from zero to its maximum; in general this time is briefer the more rapid the speed of transmission, and is extremely brief ($< 0.3 \sigma$) in the fastest motor nerves. The relation, however, does not seem to be a simple one, and other factors, such as the amplitude and duration of the electric variation, appear also to enter (cf. Blair and Erlanger, 1933). The distance (l) would depend on the special conditions of E.M.F., resistance and polarisation existing in the circuit, and is difficult to estimate or to determine experimentally. Cremer (1929) has discussed in detail the various physical factors involved and has derived a formula relating the speed to the physical conditions present in the nerve and immediate environment; his formula makes the speed proportional to the square root of the rate of development of the action potential, rather than directly to the rate, but space will not permit its discussion here (see also Gasser, 1928). An additional important factor in the speed of transmission in medullated nerves appears to be the segmentation of the myelin sheath (see below, p. 201). The general problem of the factors determining speed is probably best approached by a mathematical analysis of a simplified type of model (cf. Rashevsky, 1931, 1933); a satisfactory theory so derived would give definite direction to further experimentation.

VIII. REFRACTORY PHASE: RHYTHM

The manner in which the speed of transmission in the iron wire, as well as the responsiveness to electrical activation, varies with the interval elapsed since a previous activation is a phenomenon of special interest, since a closely similar variation of properties is characteristic of irritable living tissues during the period of recovery (the relative refractory period) immediately following a response to stimulation (cf. Brücke, 1930, for review). The iron wire exhibits, in fact, a clearly defined refractory phase during the interval immediately following the passage of an activation wave. A steel wire in 70 v. per cent. HNO_3 at 20° will not transmit a second activation wave through more than a few centimetres—the distance increasing with time—until a minute or more has elapsed since the previous activation (Lillie, 1920). A similar partial or decremental transmission occurs in living tissues also under various conditions of fatigue, injury, or narcosis. When complete transmissivity has finally returned in the wire, the activation wave travels at first much more slowly than in a completely recovered wire; later it regains by degrees its original speed (Lillie, 1925). In this respect also the wire resembles the nerve (Forbes, Ray and Griffith, 1923; Gasser and Erlanger, 1925). In general the duration of the refractory period in the wire increases with the concentration of acid. It shows the same division as in living tissues into “absolute” and “relative” periods, and the temperature coefficient of the recovery process is similar ($Q_{10} = ca. 3.0$) (Lillie, 1925). It is somewhat remarkable that the temperature coefficient of transmission in nerve is distinctly lower (of the order of $Q_{10} = 2$) than that of recovery (cf. Davis, 1926, for table of data); and the same is true of the iron model (Lillie, 1925). The low Q_{10} for

transmission in nerve is consistent with the view that an electrochemical process is the direct determinant of transmission, since such processes have low temperature coefficients.

The various peculiarities of the refractory phase in the wire point to some progressive change in the properties of the passivating film during the period immediately succeeding its first deposition. The evidence (especially the relation of rate of recovery to strength of acid: Lillie, 1931) indicates that at first the oxide layer is relatively thick, and hence resistant to disruption by the current of the local active-passive circuit; as time advances the solvent action of the acid reduces the film by degrees to its final or equilibrium thickness, which is possibly monomolecular. Reactivity and speed of transmission are then maximal. Further thinning is automatically prevented in strong acid, for the reason that any small area where the metal is exposed is a local anode of high current density where the film is automatically repaired by reoxidation (Lillie, 1929*a*). The film is thus maintained in its final state, like the plasma membrane of a living cell or axone, by a process of repair or maintenance which acts self-regulatively or automatically. The nature of the reparative process in a living nerve during the relative refractory period is not definitely known, but it appears to consist in the replacement or resynthesis of some structure or material which is broken down during excitation. Oxidative metabolism is evidently a factor of primary importance, as is shown by the indispensability of oxygen to recovery (Gerard, 1932; Hill, 1932; Cohen and Gerard, 1933); and the presence of inorganic salts, especially of calcium, seems also to be essential, indicating that an important part of the process is the return of certain altered colloidal components to their previous physical state.

The duration of the refractory period varies in different kinds of wire, and in steel wires is unusually prolonged (Lillie, 1920, 1931). In pure iron (Armco), under the conditions just defined (70 v. per cent. HNO_3 at 20°), the absolute refractory period is brief (1 sec. or less), and because of this rapid recovery, wires of this metal show certain peculiarities, especially the property of automatic regular rhythm, which are absent in steel wires (Lillie, 1928, 1929). If, for example, we place a short length of pure iron wire (2–4 cm.) in a flat-bottomed vessel containing 70 v. per cent. HNO_3 , and touch it with zinc, it will usually begin a long series of rhythmical reactions in which bright (passive) and dark (active) phases alternate regularly at a rate of 50–150 cycles per minute. Experiment shows that this rhythm is dependent on contact of the iron with the glass. One or more small local areas remain permanently active, because of depletion of acid in the narrow space between glass and wire, and these areas act as pace-making or nodal regions from which successive waves of activation travel over the whole length of the wire, at intervals determined by the duration of the refractory period. If care be taken to secure uniformity of conditions, *e.g.* by suspending the wire by thin glass filaments in the acid (kept well stirred) and inserting one end for a short distance into a narrow glass tube (to secure a pace-making area of constant extent), the rhythm is remarkably uniform and shows many other peculiarities resembling physiological rhythms. The chief of these resemblances are: (1) acceleration of rhythm by increasing the activity at the pace-

making area (*e.g.* by inserting the wire further into the tube: a parallel to the relation between intensity of stimulation and frequency of discharge (Adrian, 1932)); (2) acceleration by rise of temperature ($Q_{10} = 2.5-3$); (3) retardation or inhibition by anodal and acceleration by cathodal polarization; (4) production of rhythm in a freely suspended wire (which is typically non-rhythmical) by passing a constant current through the wire as cathode (Lillie, 1928*a*, *b*). Photographic records of the variations of potential in a pulsating wire, made with the string galvanometer, exhibit a close resemblance to the simpler types of electrocardiogram, especially of invertebrates (Lillie, 1929*a*).

The nodal or pace-making regions in rhythmical living tissues, *e.g.* the sinu-auricular node of the vertebrate heart, are regions of high automatic activity, from which waves of excitation pass over the rest of the organ, the possible rate of rhythm being limited by the duration of the refractory phase. In this respect the pulsatile wire offers a definite parallel with the living tissue. We need not assume that all protoplasmic rhythms are determined in this special manner, although control by transmission from nodal or pace-making structures is apparently a widespread condition (*cf.* Hoagland, 1935). It seems probable that factors of a more general physical kind may enter in determining the rhythms of higher frequency, such as those of sensory receptors and motor nerve cells, or of the beating of cilia, the most widely distributed rhythmical structures in living organisms. In cilia there is evidence that the basal granules, or blepharoplasts, are the pace-makers; and it is possible that in these almost submicroscopic bodies, the rhythmical recurrence of critical maxima in the impacts of adjacent reacting molecules may determine the rate of discharge, just as such impacts determine the rhythms of oscillation in Brownian movement.

IX. TRANSMISSION OF INHIBITORY INFLUENCE; POLARISATION EFFECTS (ELECTROTONUS)

Not only may states of activity be transmitted in iron wires through the influence of local circuits, as in the instances just described, but influences of the reverse type, *i.e.* repressive of activity—which we may by analogy call inhibitory influences—are also transmitted, although typically at a slower rate. A wire reacting continuously with weak HNO_3 (less than 50 v. per cent.) may be rendered passive by making it the anode in a current of sufficient intensity; anodal passivation is in fact a general method of inducing this state (*cf.* review of Bennett and Burnham, 1917). If a wire reacting in dilute HNO_3 is brought into close contact with a piece of platinum (or other noble metal), the reaction declines and then ceases, at first near the contact, and by degrees farther away, until the whole length becomes inactive; the wire is then found to be passive. The rate of travel of this influence is slow, and increases as the passive area extends. The explanation is clear; the platinum forms a cathodal area, with the adjacent iron anodal; the total oxidising, and hence passivating, influence acting on the iron is thus increased in the immediate vicinity of the platinum; then, as the iron itself becomes passive, and therefore cathodal, the same effect is repeated in the regions beyond, until the whole surface becomes passive. The view

that general electrochemical conditions of this kind are concerned in the spread of inhibitory influences in the nervous system is of course hypothetical; but it is known that diffuse or generalised inhibitory influences, such as sleep, spread slowly in the central nervous system as compared with excitatory influences (see Pavlov, 1928); and such a view is consistent with our general knowledge of the influence of electrical polarisation (anelectrotonus) on nervous activity.

Since anodal polarisation furthers passivation, its general effect is to interfere with activation, *i.e.* it retards or prevents the passage of an activation wave. An iron wire anodally polarised in nitric acid is more difficult to activate than an unpolarised wire, and transmits more slowly. This effect may readily be shown by pressing a piece of platinum foil against a wire in 70 v. per cent. acid and touching the wire at a distance with zinc; as the activation wave nears the platinum it is retarded and finally blocked; and under appropriate conditions the retardation in its speed can be observed for some centimetres before it reaches the platinum. Conversely, cathodal polarisation facilitates activation and accelerates transmission. A further analogy to nerve is that anodal polarisation shortens the refractory phase, while cathodal polarisation lengthens it (Bishop, 1927).

X. DISCONTINUOUS TRANSMISSION; INTERFERENCE; INTEGRATION

Both activating and passivating distance influences may be increased in their intensity and in their speed of transmission by certain simple modifications of arrangement. It is not necessary that the state of activity should pass continuously as a wave (or "spread") through the whole intervening stretch of wire. By simple distance-action an active area may induce activity in another area which forms part of the same circuit, even although the physical conditions for continuous spread are absent (Lillie, 1925). If, for example, we coat an iron wire (*e.g.* 25 cm. long) with paraffin, except for two short lengths (of, for example, 1 cm.) at each end, immerse it in 70 v. per cent. HNO_3 and then activate one end, the other end instantly becomes active. Or a similar experiment may be performed with two separate wires dipping into the acid at opposite sides of the vessel and connected externally through an adjustable resistance. Transmission of this type is readily explained; when one wire, or portion of wire, in the circuit is made active and therefore anodal, the other becomes automatically cathodal and is activated by distance-action if the current intensity is sufficient. The transmission of activating influence under these conditions is much more rapid than when a bare wire of the same length is used along which the activation wave passes continuously from end to end. The contrast is best observed by enclosing a wire in a narrow glass tube (2–4 mm.) filled with HNO_3 and immersing it, with the two ends projecting, in a large volume of acid; on activating one end, the other end instantly becomes active, and the two activation waves thus started pass slowly along the tube from opposite ends, meeting in the middle. Where they meet, they annul one another by mutual interference.

This mutual extinction of intersecting activation waves is apparently an effect of the mutual compensation of the oppositely oriented local circuits associated with the two waves; and similar interferences are well known in nerve and muscle. They

are perhaps most clearly illustrated in the so-called phenomenon of circus transmission, shown when a contraction wave is started in one direction in a circular ring of muscular tissue cut, for example, from a large heart (Mines, 1913; Garrey, 1914) or from the subumbrellar tissue of medusae (Mayor, 1908; Harvey, 1910). Such a single wave, under favourable conditions, will travel continually around the ring of tissue for hours or even days in one direction; but two waves, started at one point and travelling in opposite directions, invariably extinguish each other where they meet. A suspended ring of pure iron wire in a circular trough of nitric acid shows a similar phenomenon (Lillie, 1929*b*): if the ring be held at one point with platinum-tipped forceps and touched nearby with zinc the activation wave is blocked in one direction by the platinum and continues unchecked in the other direction; if the forceps are then withdrawn the "trapped wave" continues its circular motion around the ring for an indefinite period. If a second wave is started in the ring both are extinguished where they meet. The possibility that circus transmission of this type may play a part in certain unco-ordinated phenomena of transmission, such as fibrillation in the heart, has been discussed by Garrey, Mines and Lewis (cf. Lewis, 1925).

It seems likely that transmission of the discontinuous type just described, as contrasted with continuous wave-like transmission, plays an important transmitting and co-ordinating role in living organisms. In the iron wire system, arranged as above, the conditions are those of distance-action in general; *i.e.* two electrode areas, connected by a metallic conductor, are in contact with the electrolyte solution; a complete circuit must be present, with not too high resistance. The velocities of the two electrode reactions are then equal by Faraday's law; one reaction is controlled by the other. Oxidation at one electrode is associated with reduction at the other; hence if we induce a rhythmical reaction at one end of the paraffined wire just described, *e.g.* by inserting it into a glass tube, the other end pulsates in the same rhythm. Retarding the rhythm at one end, *e.g.* by touching the wire with platinum, or accelerating it, *e.g.* by increasing the pace-making area inside the tube, induces corresponding variations of rhythm at the other end of the wire. In order to secure a close correspondence in the time relations of activity at the two ends, the resistance of the circuit must be kept low; if it is increased beyond a certain point, the two areas behave independently. This synchrony in the rhythm of the two areas has its general analogies with the physical phenomenon of resonance; it should be noted that transmission of inhibitory as well as of excitatory influence is involved. The recent work of Weiss (1928, 1930, 1935) indicates that factors resembling resonance may be concerned in the selective innervations of higher animals, and it is therefore important that the possibilities of interdependent action resulting from the common position of two excitable areas in an electric circuit should be carefully considered by physiologists. Any polarisable surface situated in the path of an electric current is subjected to changes of polarisation as the current varies, and in the living cell such variations of polarisation are typically associated with variations of irritability or activity. In a complex structure like the central nervous system of higher animals it is probable that bio-electric currents are more or less continually flowing in the

interstitial spaces; the precise characters of the circuits would be determined by structural conditions, such as the disposition of conducting or non-conducting channels and layers and the other morphological or histological characters of the system. The general unity of action of such a system as the cerebral cortex may be more readily understood on this general conception, since wherever currents are free to flow they will have polarising effects and so influence activity (compare the discussion by Lashley (1931) on factors of integration in the cerebral cortex; also Gerard (1931, pp. 76 *et seq.*); cf. also the recent observations of Adrian and Matthews (1934)).

Some years ago (Lillie, 1925) I suggested that the segmented structure of medullated nerve fibres might have a definite physical relation to the high velocity of transmission in such fibres; the constrictions or nodes in the medullary sheath were regarded as representing areas where the currents of the active-resting circuits had maximal density and hence maximal stimulating effectiveness. The passive iron wire enclosed by segments of glass tubes gives a model (a variant of the arrangement already described) which shows, in fact, a greatly increased velocity of transmission. One difficulty in this conception lies in the difference in the physical characteristics of nerve and model. Although the axone is structurally continuous and is an electrolytic conductor, its specific resistance is many thousand times greater than that of the wire in the model. Hence the local currents in nerve must have low intensities; and a correspondingly high electrical sensitivity in the irritable protoplasmic filaments is required. This high sensitivity no doubt exists, and has a relation to the high speed of propagation of highly irritable nerves; it is known that in both the iron model and the nerve the speed of transmission is a direct function of electrical sensitivity (for nerve cf. Erlanger and Gasser, 1930; Blair and Erlanger, 1933). A further fact consistent with the hypothesis is the correlation which both sensitivity and conduction rate show with the diameter of the fibre. It is to be assumed that the current lines of the external or extracellular part of the active-resting circuit spread through the surrounding media; and significant correlations have been found between the electrical conductivity of these media and the velocities of propagation (Mayor, 1917; Pond, 1921). Recently Kato (1934) and his students have shown experimentally in work on single nerve fibres that the electrical sensitivity of the fibre is maximal at the constrictions or nodes, and falls off regularly with the distance from a node. Erlanger and Blair (1934) have found definite evidence of the importance of segmentation in the electrical response of whole nerves. The medullary nerve fibre behaves like a series of blocks or segments in which the current enters and leaves chiefly at the intervals between the segments. In the purely physical sense one effect of the presence of a segmented insulating sheath would be to extend a given area of polarisable or electrode-like protoplasmic surface over a greater length of nerve than would be the case in the absence of such a sheath. Whether the observed difference of velocity can be accounted for on this simple conception may perhaps be decided by the mathematical analysis of an appropriate model. Such an analysis presents great difficulties and has not been carried to completion as yet. Rashevsky, however, has shown that a simplified mathematico-physical model

regarded as consisting of two electrically conducting phases separated by an alterable and polarisable film possesses many quantitative characters similar to those of the living nerve (Rashevsky, 1931, 1933).

XI. IRRECIPROCAL TRANSMISSION

The model described above, in which two lengths of wire in contact with nitric acid are separated by a metallic connection external to the acid, has another interesting peculiarity, shown when the two lengths are unequal, namely, unequal readiness of transmission in the two directions; this inequality becomes equivalent under certain conditions to irreciprocal transmission. If one area (A) is large and the other (B) small, transmission from A to B is readier than from B to A , for the reason that although the quantities of current traversing the two electrode areas are necessarily equal, the *density* of the current is greater at the smaller surface. Hence, since electrolysis per unit area is proportional to density, the small area undergoes critical alteration at a current intensity which leaves the large area unaffected.

Irreciprocal or "one-way" transmission is a frequent phenomenon in living organisms and is especially characteristic of those cases where there is an absence of protoplasmic continuity between the interrelated elements, as in the myoneural junctions or the synapses of the central nervous system. Irreciprocal transmission may, however, be experimentally induced in tissues where the protoplasmic elements are intimately united or continuous, such as cardiac muscle, by local interference with free transmission. The essential condition appears to be the presence of some asymmetry, structural, chemical or electrical, between the regions separated; a mechanical analogy would be the action of valves. Local injury or constriction may induce irreciprocal transmission ("monodromia") in strips of cardiac muscle; the most effective method is the use of some clamping device which compresses the muscle unequally on its two sides; at a certain pressure conduction becomes irreciprocal, and the effect disappears when the pressure is released (Schmitt and Erlanger, 1928; Ashman and Hafkesbrung, 1929; Gilson, 1934). Electrical polarisation and chemical treatment, *e.g.* with KCl, may have a similar effect (*cf.* Schmitt and Erlanger).

There are a number of conditions under which activation is transmitted readily between two areas of passive metal in one direction but not in the other; these conditions may be briefly described as follows:

(1) *Differences in time factor of activation.* In observations made some years ago (Lillie, 1918) I found that passive nickel wire in HNO_3 responded less readily than passive iron wire; a longer contact of zinc was required to start an activation wave in nickel, and the local activity lasted longer and travelled more slowly. If we place a passive nickel wire in contact with a passive iron wire and activate the former, the iron instantly responds as a whole as soon as the activation wave reaches it; while, conversely, an activation wave initiated in the iron stops short at the nickel without transmitting activation. In the latter case the current passing between the iron and the nickel is too brief to activate the slower metal.

(2) *Differences in state of recovery.* This case is somewhat closely related to the

foregoing; *e.g.* a temporary irreciprocity is shown in the transmission between a steel wire, which recovers slowly, and a pure iron wire, which recovers rapidly. If the two wires, after complete recovery, are placed with their ends in contact in a trough of 70 v. per cent. HNO_3 , activation passes readily between them in either direction, and both show the full "all or none" response. But if the experiment is repeated within a few seconds, it is found that although a wave started in the pure iron wire travels rapidly along its whole length, it fails to activate the steel wire as a whole, although it may penetrate for a few centimetres. On the other hand, any wave started in the steel wire, if it travels far enough to reach the pure iron wire, activates the latter completely. In this case the irreciprocity depends on unequal rates of recovery; the steel transmitting partially or decrementally at a time when the pure iron transmits completely.

(3) *Differences in density of currents at the two electrode areas.* In the arrangement described above, in which two passive lengths of wire, connected through an adjustable resistance, are immersed in HNO_3 , no irreciprocity in the distance-action transmission is shown so long as the two lengths of wire are equal and the external conditions are symmetrical. But if we introduce into the system almost any kind of asymmetry affecting the conditions at the two electrodes, such as differences of temperature, composition of solution, surface area of metal, or electrical polarisation, transmission is found to occur with unequal readiness in the two directions. If one wire (*A*) is more readily activated than the other (*B*), we can adjust the resistance so that activation of *B* activates *A* while activation of *A* leaves *B* unchanged. Recently I have studied in some detail the relation of electrode area to distance activation. The two wires (5 cm. apart in HNO_3 of 65 v. per cent. at 22°) were protected with paraffin except at their ends. One of the exposed lengths (*A*) was kept constant at 8 cm.; the other (*B*) was varied from 4 to $\frac{1}{2}$ cm.; in the latter case the area of wire *A* was sixteen times that of wire *B*. If, under these conditions, with both wires fully recovered and the minimal resistance in the circuit, either wire is directly activated (by a brief touch with zinc) the other wire also responds. But if resistance is added to the circuit by successive stages, irreciprocity soon appears, which increases with the resistance until ultimately transmission from *B* to *A* completely disappears while that from *A* to *B* remains unchanged. In this experiment the factors determining activation are (1) intensity (*I*) of the current through the circuit; this intensity is directly proportional to the sum of the electrode areas ($A_1 + A_2$) and to the potential between the wires, and inversely proportional to the resistance; (2) the density of the current at the surface of the wire; this is equal to I/A , *A* being the area of the electrode; and (3) the state of recovery of the wire. It is found that the minimal or critical current-density (activating density) at which a wire is activated by a brief current (in this case the current flowing temporarily between the two when either is activated) is nearly constant at a given time interval after activation; the activating density is great at first, and falls off with time along a characteristic curve as the wire recovers; *e.g.* at 1 sec. after activation this density is approximately 100 times greater than it is 2 min. later, at which time recovery is almost complete (see Table II).

Table II. *Pure iron wires used. Length of wire A, 8 cm.; of wire B, 0.5 cm.; concentration of HNO₃, ca. 65 v. per cent. Temp., ca. 22°. The resistance of the circuit formed by wires and acid (with no additional resistance in the resistance box) was ca. 3 ohms. Resistance increments of 5 ohms were added successively as indicated; the least time required for a wire to recover sufficiently to respond to the activation of the other wire (column 2) was determined by touching the latter with zinc at regular intervals until the wire responded.*

Total resistance of circuit (R) (ohms)	Minimal recovery time required for each wire to respond to activation of the other wire		Relative intensities of current ($3/R \times 100$)	Relative densities ($I/A \times \text{const.}$)	
	0.5 cm. length (B)	8 cm. length (A)		Wire B (D_B)	Wire A (D_A) ($= D_B - 16$)
3	< 1 sec.	4 sec.	100	100	6.25
8	1 "	12 "	37.5	37.5	2.35
13	2 "	22 "	22.1	22.1	1.4
18	3 "	85 "	16.5	16.5	1.0
23	4 "	4 min. +	13.0	13.0	0.8
33	4-6 "	20 min. or more	9.1	9.1	0.57
43	8 "	> 35 min.	7.0	7.0	0.44

(The discrepancy between A at density 6.25 and B at almost the same density (7.0) is probably to be referred chiefly to error in the estimate of the relative areas.)

Table II gives the results of a typical series of experiments. It will be noted that the smaller area (B) always responds much sooner than the larger area; *i.e.* with each intensity of current there is a period during which activation is transmitted from A to B but not from B to A. This period becomes longer as the intensity decreases, until at a certain intensity only one-way transmission is possible. If the activating densities for both wires are plotted (as ordinates) against the durations of recovery, the time course of recovery may be traced; the points in such a graph, while somewhat scattered, fall along a continuous curve convex to the time axis, resembling closely in its general form the recovery curve of an irritable living tissue during the relative refractory period.

Irreciprocity of transmission may thus be based on the relative areas of the two electrode surfaces through which the current of the circuit flows. The current lines converge at the smaller electrode and spread out at the larger; hence, since local density is what determines activating effectiveness, the critical change initiating excitation occurs more readily at the smaller area. A converging current is intensified in its physical effect as it converges; this is readily demonstrated in irritable living tissues by passing the stimulating current through a triangular trough containing Ringer's solution and provided with electrodes at its apex and base; a current of a certain intensity will stimulate a muscle or nerve placed near the apex while it has no effect on the same preparation placed near the base. The use of fluid electrodes (of the Keith Lucas or Rushton type) is based on this principle.

(4) *Asymmetry in polarisation and other conditions.* Irreciprocity in iron wires may also be based on asymmetrical conditions of other types. Thus with wires of

equal length, using the same arrangement as above, a polarising current of moderate intensity, insufficient by itself to activate either wire, may prevent transmission from the cathodal to the anodal wire during the flow of current. When the current is broken, the former symmetry returns. Or the two wires may be made unequally susceptible to activation by rendering the durations of recovery unequal, or by changing the composition or concentration of the solution in contact with one wire. In all such cases, transmission is readier in the one direction than in the other.

XII. GENERAL CONSIDERATIONS

It would be premature to draw detailed conclusions with reference to the physiological conditions determining irreciprocity in reflex arcs and the other conducting paths in living organisms. We may, however, point out certain general possibilities. There may exist special structural conditions in the central nervous system which determine the points of entry or convergence of bio-electric currents, *e.g.* at special nodes or regions in the neuropile of the cerebral cortex (for properties of neuropile cf. Herrick, 1933, 1934). Possibilities of interaction and control, both excitatory and inhibitory, between separate regions may thus be present which are independent of the continuous transmission of impulses between them along conducting tracts. Such a type of transmission would be similar to that characteristic of medullated nerve fibre, according to the conception outlined above, where the chief entry of bio-electric currents is at the nodes; at these regions the density, and hence the stimulating effectiveness, of these currents is at a maximum. Saltatory transmission of this kind, which is imitated in the iron wire enclosed by segments of glass tubes, would be a special case of the general condition just defined. We may assume that in the nervous network of higher animals there is a general spread of current through the intercellular spaces when any local area is activated; but that this current will have its effect on other cells at a distance only when the structural conditions allow a certain critical density of current to be attained at those regions. Such regions would be regions of convergence, or nodal areas (cf. Sherrington, 1934); and their responsiveness and other properties might vary within a wide range in correlation with variations of local chemical, structural, or electrical conditions. A more complete specification of such conditions is hardly possible at present, and indeed is unnecessary; they are conditions of a general nature, such as the experiments with the iron model show may exist in systems composed of structural elements in which the critical reactions occur in thin surface layers possessing a high sensitivity to variations of electrical polarisation.

The whole comparison drawn in this review is based on the general resemblance between the passivating surface film in the iron model and the protoplasmic surface film or plasma membrane in the irritable living system (for a more detailed comparison cf. Lillie, 1919). In both cases the P.D. across the surface depends on the physical state of the film. In its resting or passive state this film is highly impermeable to ions and water-soluble molecules, and becomes permeable during activity. Local variations of permeability involve variations of potential, with the production

of local currents; these produce electrochemical reactions which in turn alter the state of the film at a distance. Transmission, inhibition, and variations of excitability are manifestations of this interdependence. The problem of the physical and chemical constitution of the plasma membrane, and of the relation of this constitution to permeability, is thus seen to be the fundamental problem underlying the general physiology of stimulation (for permeability cf. Höber, 1926, 1927; Gellhorn, 1929).

The law of polar activation, which applies to both systems, is based on a general electrochemical condition. In the iron system oxidation occurs at anodal regions, reduction at cathodal regions, and the two electrochemical processes have opposite effects on the properties of the surface film. Similarly, in the irritable protoplasmic system the polar influence is all-important. It has sometimes been pointed out that the apparent directions of the local current are reversed in the iron model as compared with the nerve. This is true only if we consider the iron core as corresponding to the protoplasm, and the HNO_3 to the external medium. It should be remembered, however, that if we consider the surface of the living cell or nerve fibre as an electrode surface, the region corresponding to the anode in the iron model would be the region where the positive current passes from the inner membrane surface to the adjacent layer of protoplasm; oxidation should be promoted at this region, and reduction at the region where the direction of current in relation to the membrane is reversed. Stimulation at the cathode, according to this conception, would be the effect of an electrochemical reducing action; while recovery should have a special dependence on oxidation processes. The law of polar excitation is consistent with this conception, as is also the general experimental fact that the presence of free oxygen is an essential condition for recovery in most irritable tissues after stimulation. Anodal polarisation, which theoretically should promote oxidation, is also favourable to recovery in injured or depressed nerve, as shown by Woronzow (1924); while cathodal polarisation has the reverse effect. On a strictly electrochemical hypothesis of stimulation and recovery, the opposite physical effects of anode and cathode would point to the special importance of some compound having a low oxidation-reduction potential, and also having strongly contrasted physical properties—such as water-solubility or adsorbability—in the oxidised and reduced states. That the presence of special reactive substances in the plasma membrane is necessary for normal irritability is indicated by the general phenomena of fatigue; and certain recent experiments of Osterhout and Hill (1933) lend further support to this view. They find that *Nitella* cells lose their responsiveness to electrical stimulation after prolonged immersion in distilled water; if then the cells are treated with the water extract of normal cells they regain their irritability. Certain other materials (including blood, adrenalin, guanidine, NH_4 compounds) have a similar restorative effect (Osterhout, 1935). According to their suggested explanation, irritability depends on the continual metabolic production of some special compound (called *R*); this substance accumulates in the plasma membrane, and its rate of formation may be influenced by chemical or other treatment. Apparently *R* is broken down by the stimulating current and restored during recovery. Such a view is consistent with the general conception, supported definitely (if indirectly) by the phenomena of the iron model, that electro-

chemical reactions in the polarisable surface layers of the irritable protoplasmic system form the primary events which underlie and determine stimulation and transmission (for general reviews of the metabolism of nerve cf. Winterstein, 1929; Gerard, 1932. For the electrical phenomena of nerve cf. Schaefer, 1934).

XIII. SUMMARY

The phenomena of activation and transmission in the passive iron wire model are described, and the various parallels with the irritable living system, especially nerve, are discussed. In general, the similarity of behaviour is to be referred to a single structural feature common to both systems, namely the presence of a thin, polarisable and chemically alterable interfacial layer or surface film (oxide film; plasma membrane) situated at the boundary between the metal, or the protoplasm, and the surrounding medium. This film undergoes characteristic changes of chemical composition and physical properties, *e.g.* of permeability and electrical polarisation, when traversed by an electric current of an intensity and duration sufficient to produce a certain critical degree of chemical decomposition. The activity of the system as a whole is controlled by the electrochemical oxidations and reductions occurring in the film under these conditions. Hence both the model and the living system are electrically sensitive and transmit local states of activity, local changes on the film being associated with local electric circuits which have electrochemical effect at regions beyond. Hence, also, the essential conditions under which electrical activation occurs are the same in both systems (polar activation, intensity-duration relationship, etc.). Activation and transmission are similarly affected in both by changes of temperature, by variations in the composition of the medium, by electrical polarisation (analogy to electrotonus), and by surface-active compounds (analogy to narcosis). Closely analogous processes of progressive recovery occur in both systems after the passage of an activation wave (refractory phase). Other resemblances are seen in the phenomena of automatic rhythm, the mutual interference of activation waves, the transmission of inhibitory influence, irreciprocal transmission, and distance influence, excitatory and inhibitory. Biological analogies of a more general kind, relating to mutual interdependence between processes occurring in spatially separated regions traversed by the same electric current—a possible factor in certain types of integration—are briefly discussed.

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ÜBER DEN GEHÖRSINN DER FISCHE

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EINLEITUNG.

Noch vor kurzer Zeit war die Mehrzahl der Zoologen, Physiologen und Ohrenärzte überzeugt, dass die Fische nicht hören können. Diese Ansicht war durch theoretische Überlegungen und experimentelle Erfahrungen begründet. *Theoretisch* wurde ein Hörvermögen bezweifelt, weil dem Labyrinth der Fische eine "Schnecke" mit ihrer Basilarmembran, die bei den höheren Wirbeltieren als Gehörorgan dient, vollständig fehlt. Auch hielt man die Fische für stumm, ein Hörvermögen hätte daher für sie keine biologische Bedeutung. *Experimentell* wurde gefunden, dass sie auf Töne und Geräusche nicht reagieren. Doch in dieser Hinsicht kamen verschiedene Forscher zu widersprechenden Ergebnissen. Heute wissen wir die Ursachen der negativen Befunde und kennen die Methoden, um positive Resultate zu erzielen. Es wird zweckmässig sein, zuerst über die Tatsachen zu sprechen und zuletzt über die Theorie.

Ich will bei dem Bericht über die Versuche zwei Fragen auseinanderhalten: erstens, *ob die Fische auf Töne und Geräusche reagieren*, und wenn diese Frage in

positivem Sinne beantwortet ist, zweitens, ob ein echtes Hörvermögen vorliegt, oder ob die Befunde durch einen gut entwickelten Tastsinn erklärt werden können.

Von den älteren Arbeiten, die Parker (1918) zusammengestellt und kritisch besprochen hat, werde ich nur die wichtigsten erwähnen.

I. REAGIEREN DIE FISCH E AUF SCHALLREIZE?

(1) Versuche mit positivem Ergebnis

(a) *Spontane Reaktionen.* Die ersten kritischen und sorgfältigen Beobachtungen über Reaktionen der Fische auf Schallreize verdanken wir dem Physiker Zenneck in Deutschland und dem Zoologen Parker in den Vereinigten Staaten.

Zenneck (1903) machte seine Versuche in der freien Natur. Er brachte unter Wasser eine Klingel an, die er von einer Brücke aus durch einen Kontakt in seiner Tasche, also optisch unbemerkt, in Tätigkeit setzen konnte. Weissfische (*Leuciscus rutilus*, *L. dobula*, *Alburnus lucidus*), die in der Nähe der Klingel waren, schwammen bei ihrem Ertönen blitzschnell davon. Die mechanischen Schwingungen, die synchron mit dem Anschlagen des Klöppels entstehen (2–3 pro Sekunde), waren durch einen Blechmantel um die Glocke ausgeschaltet, sodass nur die Tonschwingungen (mit höherer Frequenz) wirksam sein konnten.

Parker (1903, 1904) machte seine Beobachtungen an Fischen in Aquarien. *Fundulus heteroclitus* beantwortete durch Flossenbewegungen den Ton einer Bassaite oder einer Stimmgabel, der direkt auf eine Holzwand des Beckens übertragen wurde. Ähnliche Reaktionen beschrieben seine Schüler Bigelow (1904) und Manning (1924) beim Goldfisch (*Carassius auratus*). Weniger überzeugend sind Parkers Versuche (1910) an *Ammocoetes*, *Mustelus canis* und *Cynoscion regalis*; denn hier wurden die Töne durch Schläge eines schweren Pendels gegen eine Holzwand des Aquariums erzeugt; es ist nicht klar, ob der durch den Schlag erzeugte Wasserstoss oder die folgenden Schallwellen den wirksamen Reiz gebildet haben.

Bei den bisher erwähnten Versuchen war die Schallquelle im Wasser angebracht oder mit einer Wand des Behälters direkt verbunden. Aber Maier (1909) sah einen Zwergwels (*Amiurus nebulosus*) regelmässig in ein Versteck des Aquariums fliehen, wenn er nur leise mit dem Mund pfiß. Krausse (1918) hat diese Angaben bestätigt, Parker und van Heusen (1917) und Stetter (1929, S. 359) haben Ähnliches beobachtet. Haempel (1911) versenkte in ein grosses Aquarium mit Zwergwelsen eine unten geschlossene, oben offene Blechröhre, in deren luftgefüllten Innenraum von oben eine Glocke hinein hing. Das Tönen der Glocke veranlasste die Zwergwelse zu Fluchtbewegungen. Auf Schallreize, die nicht direkt auf das Wasserbecken, sondern durch die Luft—daher mit geringerer Energie—übertragen wurden, sind spontane Reaktionen bisher nur bei *Amiurus* beschrieben worden, für diesen aber von fünf verschiedenen Autoren. Der Zwergwels ist also für Schallreize sehr empfindlich.

(b) *Methode der bedingten Reflexe und Dressurversuche.* Die Beobachtung der spontanen Reaktionen ist unbefriedigend. Die Resultate sind oft undeutlich oder negativ (vgl. S. 215). Darüber muss man sich nicht wundern. Denn die Töne von Stimmgabeln und anderen Musikinstrumenten haben für die Fische keine bio-

logische Bedeutung. Eine wesentliche Verbesserung der Resultate kann man dadurch erzielen, dass man den Tönen eine biologische Bedeutung gibt, indem man mit dem Darbieten des Tones eine Belohnung (Fütterung) oder eine Bestrafung (elektrischer Schlag) verbindet.

Solches hat zuerst Meyer (1909) versucht. Er hat Goldfische so abgerichtet, dass sie beim Ertönen einer Glocke einen bestimmten Futterplatz aufsuchen. Seine kurze Mitteilung enthält keine näheren Angaben über die Durchführung. Es bleibt unklar, ob er auf die Fehlerquellen genügend geachtet und ob er seine Fische wirklich auf den Ton, oder—unabsichtlich—auf einen Begleitreiz abgerichtet hat.

Meyers Veröffentlichung ist ziemlich unbekannt geblieben. Ohne von ihr zu wissen und auch ohne gegenseitige Kenntnis der Versuche haben später McDonald (1922), Westerfield (1922), v. Frisch (1923) und Froloff (1925 und 1928) einen ähnlichen Weg mit Erfolg eingeschlagen.

Froloff arbeitete nach Pawlows Methode der bedingten Reflexe. An der Rückenflosse des Fisches wird eine Klemme befestigt, von der ein dünner Draht zu einem Schreibhebel über dem Aquarium führt. Der Fisch hängt also am Draht und seine Bewegungen werden auf einem Kymographion verzeichnet. Als Tonquelle dient ein Unterwassertelephon oder eine Klingel über dem Wasser. Einige Sekunden nach dem Erklängen des Tones erhält der Fisch einige elektrische Schläge, auf die er mit Fluchtbewegungen antwortet. Nach 5–30 solchen Versuchen reagierten die Fische schon auf den Ton allein mit Fluchtbewegungen, die vom Kymographion objektiv verzeichnet wurden. Sie müssen also die Töne wahrgenommen haben. Am besten brauchbar zu solchen Experimenten waren von Süßwasserfischen: *Perca fluviatilis*, *Acerina cernua*, *Tinca vulgaris*, *Carassius carassius*; von Seefischen: *Gadus morrhua*, *G. aeglefinus*, *Cottus scorpius*, *Corvina nigra*, *Crenilabrus griseus*, *Cr. pavo*.

McDonald (1922) benutzte, wie Parker bei seinen alten Versuchen, ein Aquarium mit darüber gespannter Saite. Die Längswände des Beckens waren aus Glas, die Querwände aus Holz. An diesen war die Saite befestigt. Die Fische (*Pimephales notatus*, ein Cyprinide) wurden täglich gefüttert und gleichzeitig die Saite zum Tönen gebracht. Eine Pappwand über dem Becken verhinderte, dass sie die schwingende Saite oder den Beobachter sehen konnten. Nach 2 Wochen hatten sie gelernt, auf das Erklängen des Tones an die Oberfläche zu kommen und hier das Futter zu erwarten. Mit einer ähnlichen Versuchsanordnung gelang Westerfield (1922) eine Tondressur bei *Umbra limi*.

Angeregt durch die wiederholten Angaben über spontane Reaktionen von *Amiurus nebulosus* auf Töne (s. S. 211) versuchte ich (v. Frisch, 1923) einen Zwergwels, dem ich zur Ausschaltung aller optischen Fehlerquellen beide Augen exstirpiert hatte, auf Mundpfeiff zu dressieren. Der Fisch lag meist ruhig in einem Versteck seines Aquariums. Ich fütterte ihn täglich und pfiff dabei einigemal mit dem Mund. Vom sechsten Tag ab reagierte er auf den Pfeiff, noch bevor das Futter in das Aquarium gebracht wurde. In dreissig folgenden Versuchen versagte er nicht ein einziges Mal und kam durchschnittlich 5 Sekunden nach Beginn des Pfeifens aus seinem Versteck heraus zur Wasseroberfläche, gleichgültig, ob ich laut oder leise,

unmittelbar neben dem Becken oder in einer Entfernung von mehreren Metern, pfiß. Auch bei einem zweiten blinden Wels führte solche Dressur schnell zum Erfolg.

Mein Schüler Stetter (1929) hat diese Versuche fortgeführt und weiter ausgedehnt. Auf seine sorgfältige Arbeit werde ich noch mehrmals zurückkommen. Hier sei nur erwähnt, dass sich alle von ihm geprüften Fischarten auf Töne dressieren liessen (*Amiurus nebulosus*, *Phoxinus laevis*, *Idus melanotus*, *Carassius auratus*, *Cobitis barbatula* und *Cottus gobio*). Am besten bewährten sich die Elritzen (*Phoxinus laevis*). Die Fische waren durch Exstirpation der Augen geblendet. Dies ist die einfachste Methode, um optische Fehlerquellen zuverlässig auszuschalten¹. Bei der Dressur wurde ein Ton erzeugt und gleichzeitig dem Fisch an einem Futterstab ein kleiner Fleischbrocken gereicht. Nach etwa 5–20 Dressurfütterungen² reagieren die Tiere schon auf den Ton allein durch Such- und Schnappbewegungen. Sie stoppen ihr Schwimmen plötzlich ab und suchen das erwartete Futter. Bei den weiteren Versuchen wird nach dem Darbieten des Tones immer erst einige Sekunden gewartet, ob der Fisch reagiert, und dann erst das Futter gereicht. So ist jeder Versuch zugleich eine Fortsetzung der Dressur. Als Tonquellen benützte Stetter hauptsächlich Stimpfpeifen, Edelmannpeifen und Stimmgabeln. Während die Pfeifentöne durch die Luft übertragen wurden, sind die Stimmgabeln hierfür zu leise. Sie wurden nach dem Anschlagen neben dem Becken auf den Tisch gesetzt, wobei der kleine, aber unvermeidliche Stoss beim Aufsetzen durch eine Filz- oder Gummiunterlage abgeschwächt wurde. Kontrollversuche mit nicht angeschlagenen Stimmgabeln zeigen, ob der Fisch auf die Schallwellen oder auf den Stoss des Aufsetzens reagiert. Wenn letzteres vorkommt, kann man es ihm abgewöhnen, indem man häufig die "stumme Gabel" aufsetzt ohne Futter zu reichen. Stetter hat in seinen (mehreren tausend) Versuchen so klare Reaktionen erzielt, dass kein Zuschauer an ihrer Realität gezweifelt hat. Wichtig ist, dass die Fische gesund und fresslustig sind.

Eine Reihe von Untersuchungen der letzten Jahre brachte Bestätigungen aus England, Amerika, Ungarn, Holland und Deutschland. Bull (1928, 1930) gelang eine Dressur von *Crenilabrus melops* und von *Anguilla vulgaris* auf Futter- und Schreckreize, wobei die Töne direkt auf das Wasser übertragen wurden. Moorhouse (1933) gibt an, dass *Cymatogaster aggregatus* einen lauten, im Wasser erzeugten Hornton mit der Fütterung assoziierte. Farkas (1934, 1935) fand bei *Lebistes reticulatus* nach Dressur auf Futter ausgezeichnete Reaktionen; er verwendete zur Tonerzeugung elektrische Apparate, die Töne wurden durch die Luft oder direkt auf das Wasser übertragen; der Beobachter befand sich meist in einem Nebenzimmer, von wo aus auch der Tonapparat bedient werden konnte. Benjamins (1934) wiederholte die Dressurversuche an Elritzen. In unserem Institut hat

¹ Farkas (1934) verwirft das Blenden der Fische, weil "die Enukleation der Augen die mit dem Acusticusgebiet zusammenhängenden Nerven beeinflusst". Aber die von Stetter und mir geblendeten Fische haben Monate und Jahre nach der Blendung (beobachtet bis 2½ Jahre) ausgezeichnet auf Töne reagiert. Also hat die Augenexstirpation ihr Hörvermögen nicht geschädigt. Auch Farkas hat, nach brieflicher Mitteilung, keinen nachteiligen Einfluss der Blendung festgestellt, sondern ihn nur vermutet.

² Hafen (1935) hat an Elritzen gezeigt, dass nicht die Zahl der Dressurstage, sondern die Zahl der Dressurfütterungen massgebend ist. Man kommt also schneller zum Ziel, wenn man mehrmals am Tage mit kleinen Futterbrocken, als wenn man selten und mit grösseren Brocken futtert.

sich Denker (1931) an Elritzen (*Phoxinus laevis*), Goldfischen (*Carassius auratus*), und Goldorfen (*Idus melanotus*) von ihren guten Reaktionen auf Pfeifentöne überzeugt und v. Boutteville (1935 und weitere, noch nicht veröffentlichte Versuche) mit Pfeifentönen und einem Otoaudion an *Phoxinus laevis*, *Hemigrammus caudovittatus*, *Hyphessobrycon flammeus*, *Pyrrhulina rachoviana*, *Gymnotus electricus* und *Macropodus viridiauratus* positive Ergebnisse erzielt. Bei *Umbra pygmaea* fanden v. Frisch und Stetter (1932, S. 795–6) eine gute Reizbeantwortung nur bei direkter Übertragung der Töne auf die Wand des Aquariums.

Mit der Dressurmethode erhält man weitaus bessere und zuverlässigere Resultate als durch die Beobachtung spontaner Reaktionen. Ihre Überlegenheit geht auch daraus hervor, dass, trotz vieler Untersuchungen, *Spontanreaktionen* auf Schallreize bei Luftübertragung bisher nur bei *einer* Fischart (*Amiurus nebulosus*) beobachtet wurden, während die Dressurmethode auch bei Luftübertragung schon bei *dreizehn* Arten zu positiven Ergebnissen führte.

Im Folgenden bringe ich eine *Liste aller Fische, bei welchen Reaktionen auf Schallreize zuverlässig festgestellt worden sind*. Die ersten 4 Familien werden als *die Ostariophysen* zusammengefasst¹. Bei ihnen steht die Schwimmblase mit dem Labyrinth durch die Weberschen Knöchelchen in Verbindung. Auf die Bedeutung dieser anatomischen Besonderheit komme ich später zurück (S. 233). Ein Sternchen vor dem Namen bedeutet, dass bei dieser Art auch *spontane* Reaktionen auf Schallreize beobachtet wurden. *Kursivdruck* bedeutet, dass bei dieser Art Reaktionen auch bei Luftübertragung beobachtet wurden².

I. Ostariophysen

Familie	Art	Autor
CHARACINIDAE	<i>Hemigrammus caudovittatus</i> <i>Hyphessobrycon flammeus</i> <i>Pyrrhulina rachoviana</i>	v. Boutteville, 1935 " "
CYPRINIDAE	* <i>Leuciscus rutilus</i> * <i>L. dobula</i> * <i>Alburnus lucidus</i> * <i>Carassius auratus</i> <i>C. carassius</i> <i>Tinca vulgaris</i> <i>Pimephales notatus</i> <i>Phoxinus laevis</i>	Zenneck, 1903 " Bigelow, 1904; Manning, 1924 Froloff, 1925 Froloff, 1925, 1928 McDonald, 1922 Stetter, 1929; Denker, 1931; v. Frisch und Stetter, 1932; Benjamins, 1934; v. Boutteville, 1935; Hafen, 1935 Stetter, 1929; Denker, 1931 Stetter, 1929; Denker, 1931 Stetter, 1929
	<i>Idus melanotus</i> <i>Carassius auratus</i> <i>Cobitis barbatula</i>	
GYMNOTIDAE	<i>Gymnotus electricus</i>	v. Boutteville, 1935
SILURIDAE	* <i>Amiurus nebulosus</i>	Maier, 1909; Haempel, 1911; Parker und van Heusen, 1917; Krausse, 1918; v. Frisch, 1923; Stetter, 1929

¹ Mit wenigen Ausnahmen sind alle Ostariophysen Bewohner des Süßwassers. Nahezu drei Viertel aller bekannten Süßwasserfische sind Ostariophysen.

² Unter sonst gleichen Bedingungen werden Schallwellen, die durch die Luft an das Becken herangetragen werden, mit geringerer Energie zum Fisch gelangen als Schallwellen, die direkt auf das Becken oder in das Wasser übertragen werden. Reaktionen auf Schallreize bei Luftübertragung können daher als ein Hinweis auf grössere Empfindlichkeit betrachtet werden.

II. Nicht-Ostariophysen

Familie	Art	Autor
ESOCIDAE	<i>Umbra limi</i> <i>U. pygmaea</i>	Westerfield, 1922 v. Frisch und Stetter, 1932
CYPRINODONTIDAE	* <i>Fundulus heteroclitus</i> <i>Lebistes reticulatus</i>	Parker, 1904 Farkas, 1935
ANGUILLIDAE	<i>Anguilla vulgaris</i>	Bull, 1928
OSPHROMENIDAE	<i>Macropodus viridiauratus</i>	v. Boutteville, noch nicht veröffentlicht
SCIAENIDAE	<i>Corvina nigra</i>	Froloff, 1925
PERCIDAE	<i>Perca fluviatilis</i> <i>Acerina cernua</i>	Froloff, 1925 Froloff, 1925
EMBIOTOCIDAE	<i>Cymatogaster aggregatus</i>	Moorhouse, 1933
LABRIDAE	<i>Crenilabrus griseus</i> <i>C. pavo</i> <i>C. melops</i>	Froloff, 1925 Froloff, 1925 Bull, 1928
COTTIDAE	<i>Cottus scorpius</i> <i>Cottus gobio</i>	Froloff, 1925 Stetter, 1929
GADIDAE	<i>Gadus morrhua</i> <i>G. aeglefinus</i>	Froloff, 1925 Froloff, 1925

Reaktionen auf Schallreize wurden also an 32 Arten aus 14 verschiedenen Familien zuverlässig festgestellt. Nur bei völliger Unkenntnis der Literatur kann man noch behaupten, dass Fische auf Schallreize nicht reagieren.

(2) Versuche mit negativem Ergebnis

Nach dieser Übersicht haben die negativen Befunde mancher Forscher, die früher zu so lebhaften Kontroversen geführt haben, vorwiegend historisches Interesse. Aber es ist auch lehrreich, den Ursachen ihrer Misserfolge nachzugehen.

Einen Grund für negative Ergebnisse habe ich schon erwähnt: Singen, Pfeifen, Glocken- und Stimmgabeltöne sind für die Fische primär ohne biologische Bedeutung. Daher kann man nicht erwarten, dass sie von vornherein zuverlässig darauf reagieren. Es ist also nicht erstaunlich, dass den positiven Beobachtungen über Spontanreaktionen (S. 211) auch viele Angaben über das *Ausbleiben spontaner Reaktionen* gegenüberstehen (z. B. Kreidl (1895) für *Carassius auratus*, H. N. Maier (1909) für *Gadus morrhua*, *Clupea harengus*, *Ammodytes lanceolatus*, *Trigla gunardus*, *Cottus scorpius*, *Rhombus maximus*, *Solea vulgaris*, *Cyprinus carpio*, *Alburnus lucidus*, *Idus melanotus*, *Gobio fluviatilis*, *Anguilla vulgaris* u. a., so auch Körner (1905, 1916, 1919) und Lafite-Dupont (1907) für verschiedene Knochenfische, Bernoulli (1910) für *Salmo fario*, *Lucioperca sandra* und *Anguilla vulgaris*, Haempel (1911) für *Cyprinus carpio*, *Scardinius erythrophthalmus*, *Gobio fluviatilis*, *Trutta fario*, du Bois-Reymond (1917) für Barsche, Moorhouse (1933) für einen Hai, einige Pleuronectiden und Cottiden).

Aber es liegen auch Angaben vor über das *Misslingen von Dressuren*. Hierher gehört die oft zitierte Beobachtung von Kreidl (1896) im Benediktinerkloster Kremsmünster. In einem Fischteich wurden Forellen (*Salmonidae*) durch Läuten

einer Glocke zur Fütterung gerufen. Sie kamen tatsächlich, wenn die Glocke geläutet wurde. Aber Kreidl zeigte, dass sie optisch und nicht akustisch angelockt wurden. Bull (1928) versuchte bei *Blennius gattorugine* (Blenniidae) vergeblich eine Futterdressur auf die Töne eines Unterwassertelephons, bei *Gasterosteus aculeatus* (Gasterosteidae), *Cottus bubalis* (Cottidae), *Gobius minutus* (Gobiidae), *Pleuronectes platessa* (Pleuronectidae) vergeblich eine Schreckdressur mit elektrischen Schlägen, wobei ein ins Wasser getauchter Summer als Schallquelle diente. Denker (1931) konnte einen Flussbarsch (Percidae) nicht auf Pfeifentöne, Forellen (Salmonidae) weder auf Pfeifentöne noch auf Stimmgabeln dressieren. Überblickt man diese Fischfamilien, so fällt auf, dass sie alle zu den Nicht-Ostariophysen gehören. Ostariophysen haben bei Dressurversuchen auf Töne noch niemals versagt. Wir werden die Erklärung für diesen auffallenden Gegensatz in der gesteigerten Tonempfindlichkeit der Ostariophysen kennen lernen.

(3) Grenzen der Tonwahrnehmung

Die Grenzen der Tonwahrnehmung können durch die Dressurmethode zuverlässig bestimmt werden. Stetter (1929) fand mit der Edelmann- und Galtonpfeife¹ in sorgfältigen Versuchsreihen an sieben Elritzen (*Phoxinus laevis*) die obere Grenze zwischen d^5 und a^5 (4645–6960 v.d.). Die Methode gibt sehr scharfe Resultate. Dies mag die folgende Übersicht illustrieren, in der alle Versuche mit einer von den sieben Elritzen zusammengestellt sind (Dauer dieser Versuchsreihe: 6 Wochen).

Tonhöhe	Zahl der Versuche mit		
	positiver Reaktion	undeutlicher Reaktion	negativer Reaktion
$c^3 = 4138$ v.d.	19 = 100 %	0 = 0 %	0 = 0 %
$d^5 = 4645$ v.d.	49 = 74 %	3 = 5 %	14 = 21 %
$dis^5 = 4922$ v.d.	2 = 15 %	1 = 8 %	10 = 77 %
$e^5 = 5213$ v.d.	3 = 9.5 %	2 = 6 %	27 = 84.5 %

Idus melanotus reagierte noch sicher auf f^5 (5524 v.d.), *Carassius auratus* auf a^4 (3480 v.d.). Bei *Cobitis barbatula* lag die Grenze zwischen a^3 und a^4 (1740–3480 v.d.). Bei anderen Cypriniden wurde die obere Grenze der Tonwahrnehmung bisher nicht untersucht.

An einem Gymnotiden (*Gymnotus electricus*) erzielte v. Boutteville (1935) nur bis zu den Tönen a^2 – c^3 (870–1035 v.d.) zuverlässige Reaktionen, während sie an drei Characinidenarten (*Hyphessobrycon flammeus*, *Hemigrammus caudovittatus* und *Pyrhulina rachoviana*) bis zu a^5 (6960 v.d.) sehr gute Resultate erhielt.

Die besten Leistungen hat der uns schon rühmlich bekannte Zwergwels (*Amiurus nebulosus*) aus der Familie der Siluridae aufzuweisen. Stetter fand hier die obere Grenze der Tonwahrnehmung höher als gis^6 (13,139 v.d.).

Bei dem Cyprinodontiden *Lebistes reticulatus* liegt sie nach Farkas (1935) zwischen c^3 und c^4 (1035–2069 v.d.). Dies ist die einzige Angabe über die obere Grenze der Tonwahrnehmung bei einem Nicht-Ostariophysen.

¹ Über die Fehlerquellen, die bei Benützung der Galtonpfeife zu beachten sind, vgl. Stetter, 1929, S. 369.

Es bestehen also bei verschiedenen Fischarten bedeutende Unterschiede. Bei derselben Fischart kommen individuelle Abweichungen vor, die aber gegenüber den Artverschiedenheiten gering sind. Bei *Amiurus* stimmt die obere Grenze der Tonwahrnehmung (etwa 13,000 v.d.) mit der oberen Hörgrenze des Menschen angenähert überein.

Eine *untere Grenze* der Tonwahrnehmung wurde von Stetter (1929) nicht gefunden. Die Fische reagierten noch auf periodische Schwingungen von sehr geringer Frequenz. Man kann einwenden, dass sie nicht den beabsichtigten Ton, sondern einen seiner Obertöne wahrgenommen hätten. Diesen Einwand hat Stetter bei Elritzen auf zweierlei Weise widerlegt: erstens durch Verwendung von obertonfreien Stimmgabeln; zweitens konnte er durch eine Differenzdressur (s. S. 217, unten) erreichen, dass die Fische nur auf den tiefen Dressurton, nicht aber beim Darbieten der nächst höheren Oktave oder von deren Quinte die Futterreaktion gaben. Sie haben also auf die lautesten Obertöne nachweislich nicht reagiert.

Es ist somit sicher, dass die Elritze auch sehr tiefe Töne bemerkt. Es wird aber später (S. 231) zu berichten sein, dass hier der Tastsinn beteiligt ist und dass es eine untere Grenze für ihre Wahrnehmungen durch das Ohr, eine untere *Hörgrenze*, doch gibt. Sie liegt zwischen C_2 und C_1 (16–32 v.d.).

(4) Tonunterscheidung

Die Frage, ob die Fische verschieden hohe Töne voneinander unterscheiden können, ist bei dem Mangel einer Basilarmembran von besonderem theoretischen Interesse. Schon Meyer (1909) hat Goldfische darauf abgerichtet, "dass sie beim Ertönen einer hohen Glocke mit beträchtlicher Sicherheit den Weg zu einem Futterplatz einschlagen, beim Ertönen einer tiefen Glocke den Weg zu einem andern Futterplatz im Aquarium". Da alle näheren Angaben fehlen, kann man sich aber über die Zuverlässigkeit dieser Mitteilung kein Urteil bilden. *Crenilabrus melops* hat in Versuchen von Bull (1930) eine ähnliche Aufgabe nicht gelöst. Allerdings war die Versuchsanordnung nicht sehr zweckmässig, die Anforderung an den Fisch zu schwierig.

Einwandfreie Prüfungen des Tonunterscheidungsvermögens hat Stetter (1929) an Elritzen (*Phoxinus laevis*) und Zwergwelsen (*Amiurus nebulosus*) durchgeführt. Der blinde Fisch wird zunächst auf einen Futterton dressiert. Sobald er ihn mit einer deutlich ausgeprägten Futterreaktion (Suchen und Schnappen) beantwortet, wird zwischendurch ein anderer, um 1–2 Oktaven höherer (oder tieferer) Ton geboten, bei dem der Fisch kein Futter erhält. Gibt er die Futterreaktion, so wird er durch einen leichten Schlag mit einem Glasstäbchen bestraft. Durch oft wiederholtes, abwechselndes Darbieten des "Futtertones" mit folgender Fütterung (Belohnung) und des "Warntones" ohne Fütterung oder mit folgender Strafe lernt der Fisch ihre Unterscheidung (*Differenzdressur*). Er gibt dann nur mehr auf den Futterton die Futterreaktion, auf den Warnton bleibt jede Reaktion aus oder sie äussert sich in deutlich anderer Weise, z. B. durch fluchtartiges Niedergehen an den Boden. Durch allmähliche Verminderung des Tonintervalles lassen sich die Grenzen des Tonunterscheidungsvermögens feststellen. Stetter berichtet über

ausgedehnte, erfolgreiche Versuchsreihen an neun Elritzen und zwei Zwergwelsen. Die Elritzen waren für die Differenzdressuren besser geeignet als die Welse. In der Regel lernten sie die sichere Unterscheidung zweier Töne bis zu einem Intervall von etwa einer Oktave. Die beiden besten Elritzen brachten es bis zur sicheren Unterscheidung einer grossen Terz (Futterton $gis^2=821$ v.d., Warnton $e^2=652$ v.d.) und einer kleinen Terz (Futterton $d^1=290$ v.d., Warnton $f^1=345$ v.d.). Bei der Beurteilung dieser Leistungen muss man daran denken, dass Futterton und Warnton nie unmittelbar nacheinander geboten wurden, sondern in Abständen von vielen Minuten, Stunden oder Tagen. Bei seinen Gedächtnisversuchen hat Stetter (1929, S. 472) an verschiedenen Elritzen sogar noch nach Pausen von 1–9 Monaten richtige Antworten auf die Futter- und Warntöne erhalten. Die Fische haben also ein erstaunlich gutes Gedächtnis für die absolute Tonhöhe. Für Säugetiere ist bekannt, dass sie ein gutes absolutes Gehör haben. Es scheint dies eine ursprüngliche Fähigkeit der Tiere zu sein, die beim Menschen mit der Steigerung der geistigen Entwicklung teilweise verloren gegangen ist.

Stetter hat sich bei seinen Elritzen mit der Unterscheidung *zweier* Töne nicht begnügt. Bei fünf Versuchstieren gelang es ihm, die gute Unterscheidung *dreier* Töne (zwei Warntöne und ein Futterton, oder ein Warnton und zwei Futtertöne) zu erzielen. Eine Elritze lernte die *vier* Töne c^5 (4138 v.d., Warnton), e^3 (1303 v.d., Futterton), e^2 (652 v.d., Warnton), g^1 (388 v.d., Futterton) und das Geräusch einer Knarre richtig zu unterscheiden und machte in einer Versuchsreihe mit 153 Versuchen nur vier Fehler. Eine andere hat nicht so sicher, aber doch sehr gut die *fünf* Töne e^3 (1303 v.d.), e^2 (652 v.d.), d^1 (290 v.d.), e (163 v.d.) und C_2 (16 v.d.) auseinandergehalten.

Bemerkenswert ist auch die Fähigkeit der Elritzen und Welse, den Futterton beim gleichzeitigen Erklängen von zwei oder drei Tönen aus dem Klang "herauszuhören". Sie reagieren nur dann, wenn in dem Zusammenklang der Dressurton enthalten ist. Dagegen gelingt die Dressur auf die verschiedene Klangfarbe *eines* Tones, der auf verschiedenartigen Instrumenten erzeugt wird, nur sehr mangelhaft (Stetter, 1929).

Differenzdressuren an Elritzen sind seither in unserem Institut noch oft mit Erfolg ausgeführt worden.

An Nicht-Ostariophysen liegen über eine Prüfung der Tonunterscheidung nur wenige Angaben vor: der misslungene Versuch von Bull an *Crenilabrus melops* wurde oben schon erwähnt. Westerfield (1922) dressierte *Umbra limi* auf den Ton d^1 (290 v.d.), wobei die Tonquelle, ein gitarre-ähnliches Saiteninstrument, auf den Rand des Aquariums aufgesetzt und gleichzeitig Futter gegeben wurde. Als Warnton wurde a^1 (435 v.d.) geboten, wobei die Fische ein mit Kampferspiritus getränktes Stückchen Papier erhielten. Sie sollen rasch gelernt haben, dieses Intervall (eine Quinte) sehr sicher zu unterscheiden. Die Beschreibung der Versuchsanordnung erweckt aber manche Bedenken (vgl. Stetter, 1929, S. 398). Eine Nachprüfung wäre erwünscht. Farkas (1935) hat bei *Lebistes reticulatus* keine zuverlässige Tonunterscheidung erzielt. Ein Urteil über die Leistungen der Nicht-Ostariophysen kann man sich auf Grund dieser Versuche noch nicht bilden.

(5) Reizschwellen

Die ersten genauen Untersuchungen über die *Reizschwelle*—oder über die *Hörschärfe* der Fische, wie wir mit Bezug auf den folgenden Abschnitt auch sagen können—stammen wieder von Stetter (1929). Als Vergleichsmaßstab benützte er die Hörschärfe des Menschen.

Ein Aquarium mit einer blinden Elritze wurde in einem 120 m. langen Korridor aufgestellt. Sie war auf den Pfeifenton e^a (652 v.d.) ausgezeichnet dressiert. Nun wurde der Pfeifenton durch Drosselung der Luftzufuhr so weit abgeschwächt, dass er eben noch rein und klar erklang. Er hatte die Lautstärke eines ziemlich leisen Mundpiffes. In einer langen Versuchsreihe und unter steter Mitwirkung eines zweiten Beobachters wurde die Entfernung festgestellt, aus welcher der Piff eben noch eine Reaktion auslöste. Diese Grenze lag bei 60–80 m. Für den Menschen (sechs Versuchspersonen) war der Ton aus dieser Entfernung eben noch hörbar, aus einer Entfernung von 90–100 m. war er nicht mehr wahrnehmbar. Die Versuchsbedingungen waren aber für Mensch und Fisch ungleich, weil dieser im Wasser geprüft wurde und die Schallwellen beim Übergang aus der Luft ins Wasser an Intensität verlieren. Es wurden deshalb Vergleichsversuche an Menschen unter Wasser durchgeführt. Sie waren in einem grossen Aquarium untergetaucht, das an derselben Stelle stand wie das Fischbecken bei den Reizschwellenprüfungen. Die Versuchsperson kniete im Becken auf dicken Filzpolstern und war vollständig unter Wasser. Der Pfeifenton wurde bei dieser Anordnung nur bis zu einer Entfernung von 30–50 m. gehört. Die Elritze hat also den sehr leisen Ton etwas besser wahrgenommen als ein Mensch unter Wasser, und etwas schlechter als ein Mensch ausserhalb des Wassers. Diese erstaunliche Leistung konnte Stetter an zwei weiteren Elritzen und an zwei Zwergwelsen bestätigen. Es ist aber zu beachten, dass es *Bestleistungen* waren, wie sie nur unter günstigen Bedingungen erreicht werden können. Denn Stetter hat für die Versuche besonders gut reagierende Fische ausgesucht und er hat diese Experimente fast immer in der stillen Nachtzeit ausgeführt.

Meine Schülerin v. Boutteville (1935) hat die Hörschärfenversuche (gleichfalls mit dem Ton e^a) an einer Elritze wiederholt und auf Characiniden und einen Gymnotiden ausgedehnt. Sie hat teilweise, so wie Stetter, mit der abgeschwächten Edelmannpfeife gearbeitet und hierbei die ausgezeichnete Hörschärfe der Elritze bestätigt und eine ähnliche Empfindlichkeit bei *Hyphessobrycon* gefunden. Zu weiteren Versuchen benützte sie einen Überlagerungssender (Otoaudion), mit dem es möglich war, die Intensität des Tones beliebig und in kontrollierter Weise abzustufen. Der Überlagerungssender war mit einem Barkhausenapparat der Firma Siemens und Halske auf Phon geeicht. Von Characiniden prüfte sie *Hyphessobrycon flammeus*, *Hemigrammus caudovittatus* und *Pyrrhulina rachoviana*. Alle drei Arten hatten angenähert dieselbe Hörschärfe. In über 1000 Versuchen an sechs Individuen wurden bei einer Lautstärke von 21 Phon noch sichere Reaktionen erzielt, bei 17 und 15 Phon überwiegen die negativen Resultate und 13 Phon kann als Schwellenwert betrachtet werden. Um Anschluss an die Resultate von Stetter zu

gewinnen hat v. Boutteville auch Vergleichsversuche an Menschen ausgeführt. Die Reizschwelle für Characiniden lag etwas höher als für das menschliche Ohr unter natürlichen Verhältnissen (Differenz etwa 13 Phon) und angenähert gleich bei Prüfungen unter Wasser. Die Hörschärfe der Characiniden stimmt also angenähert überein mit der Hörschärfe der Elritze, des Zwergwelses und des Menschen. An *Gymnotus electricus* fielen die Prüfungen bei einer Lautstärke von 35–40 Phon noch teilweise positiv aus. Doch mussten die Versuche leider vorzeitig abgebrochen werden. Eine beträchtliche Hörschärfe ist auch hier anzunehmen.

An *Nicht-Ostariophysen* wurden Reizschwellenbestimmungen bisher nicht ausgeführt. Aber es sind durch Stetter und v. Boutteville alle vier Familien der *Ostariophysen* untersucht mit dem Ergebnis, dass die Empfindlichkeit von Cypriniden (*Phoxinus*), Siluriden (*Amiurus*) und Characiniden (*Hyphessobrycon*, *Hemigrammus*, *Pyrhulina*) für den Ton e^2 (652 v.d.) von der gleichen Grössenordnung ist wie die menschliche Hörschärfe für diesen Ton, und dass Gymnotiden (*Gymnotus*) jedenfalls nicht weit davon entfernt sind. Es ist nicht wahrscheinlich, dass die Empfindlichkeit der Fische nur für einen kleinen Tonbereich diese Schärfe hat, dass also der Ton e^2 eine Sonderstellung einnehmen würde. Ich habe mit dem Otoaudion die Hörschärfe einer Elritze für e^2 (652 v.d.) und für a^3 (1740 v.d.) geprüft und mit dem hohen Ton keine schlechteren Resultate erhalten. Schon früher hatten wir (v. Frisch und Stetter, 1932, S. 744) Gelegenheit, die grosse Empfindlichkeit der Elritzen für tiefe Töne ($C=65$ v.d., $C_1=32$ v.d.) kennen zu lernen.

Es ist schade, dass genaue Schwellenwertbestimmungen, die sich zum Vergleich auf die Hörschärfe des Menschen beziehen, bisher für die anderen Tonbereiche nicht vorliegen. Aber wer solche Versuche nicht miterlebt hat, macht sich kaum eine Vorstellung von dem grossen Aufwand an Zeit und Mühe, den sie erfordern.

II. HABEN DIE FISCH EINE GEHÖRSINN?

(1) Definition

Im vorigen Abschnitt wurde berichtet, dass Fische auf Schallreize reagieren. Wir fragen jetzt, ob sie *hören*.

Wir wissen von uns selbst, dass wir einen Ton *durch das Ohr hören*, dass wir aber denselben Ton, wenn er genügend laut und nicht zu hoch ist, auch *durch die Haut fühlen* können. Wir sprechen darum auch bei den Fischen von einem *Hörvermögen* nur dann, wenn die Töne durch das *Labyrinth* perzipiert werden. Eine Wahrnehmung von Schallwellen durch die Körperhaut fällt in das Gebiet des Tastsinnes.

Bei manchen Untersuchungen über das Hörvermögen der Fische ist besonderer Wert darauf gelegt worden, dass die Schallwellen nur durch die Luft und nicht durch den Boden an das Aquarium gelangen können. Man hat für eine erschütterungsfreie Aufstellung der Becken gesorgt. Es hat dies einen Sinn, wenn die Fische durch die Tritte der sich nähernden Personen beunruhigt oder auf die bevorstehende Fütterung aufmerksam werden. Auch muss man natürlich bei quantitativen Versuchen (Reizschwellenbestimmungen) darauf achten, ob die Schallwellen, die auf dem Luftwege übermittelt werden sollen, nicht gleichzeitig durch den Boden mit

grösserer Intensität zugeleitet werden. Aber für die Frage der *Hörfähigkeit* ist es ganz nebensächlich, ob die Schallwellen durch den Boden oder durch die Luft oder direkt auf das Wasser übertragen werden. Entscheidend ist, mit welchem Organ sie der Fisch wahrnimmt.

(2) Zur Anatomie des Fischlabrynth

Zum Verständnis des Folgenden sind einige anatomische Kenntnisse notwendig. Den Fischen fehlt ein Trommelfell und Mittelohr. Diese Erwerbungen der landbewohnenden Wirbeltiere bedeuten eine Anpassung an das Hören in der Luft. Das häutige Labyrinth der Knochenfische liegt innerhalb der Schädelkapsel, vom Gehirn nicht oder nur teilweise durch Knochenwände abgegrenzt. Seine Gestalt ist sehr variabel. Wenn wir von nebensächlichen Unterschieden absehen, können wir *zwei Typen* auseinanderhalten:

(a) *Normal-Typus*. Dem Labyrinth der meisten Fische liegt ein Bauplan zugrunde, wie er etwa bei der Forelle (*Salmo fario*) verwirklicht ist (Abb. 1)¹. Die

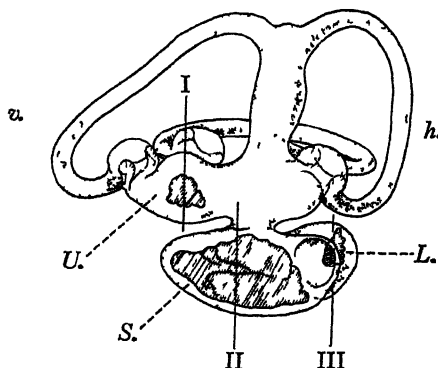


Abb. 1. Forelle (Normal-Typus).

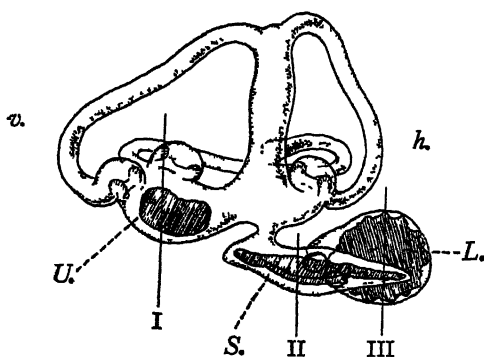


Abb. 2. Elritze (Ostariophysen-Typus).

Rechtes Labyrinth von der Innenseite. v. = vorne, h. = hinten. U. = Utriculus, S. = Sacculus, L. = Lagena. Querschnitte durch die Labyrinth entsprechend den Linien I, II und III sind in den Abbildungen 3–8 wiedergegeben.

pars superior besteht aus dem Utriculus und den drei Bogengängen. Durch eine starke Einschnürung ist die *pars inferior* von ihr abgegrenzt; sie besteht aus dem Sacculus und der Lagena. Am Boden des Utriculus und an der medialen Wand des Sacculus und der Lagena befindet sich je ein Polster von Sinneszellen. Im Utriculus ruht ein Otolith², der Lapillus, auf dieser Nervenendstelle (Abb. 3). Der Otolith des Sacculus, die Sagitta, liegt der Nervenendstelle seitlich an. Durch seine Randfasern wird er in dieser Lage festgehalten (Abb. 5). Dasselbe gilt für den Otolithen der Lagena, den Asteriscus (Abb. 7). Weitere Nervenendstellen, aber ohne Otolithen, finden sich in den drei Ampullen der Bogengänge (Cristae) und an der Öffnung zwischen Utriculus und Sacculus (papilla neglecta)³.

¹ Alle Zeichnungen sind von Herrn Dr. R. Ehrlich ausgeführt.

² Die Otolithen der Knochenfische sind feste Steinchen aus CaCO_3 .

³ Von den Cristae in den Bogengangampullen weiss man, dass sie auf Strömungen der Endolymphe ansprechen. Von der papilla neglecta ist nach ihrem Bau dasselbe zu vermuten.

Die relative Grösse der Otolithen kann bei verschiedenen Arten sehr ungleich sein. Insbesondere kann die Lagena mit ihrem Asteriscus viel grösser, aber auch

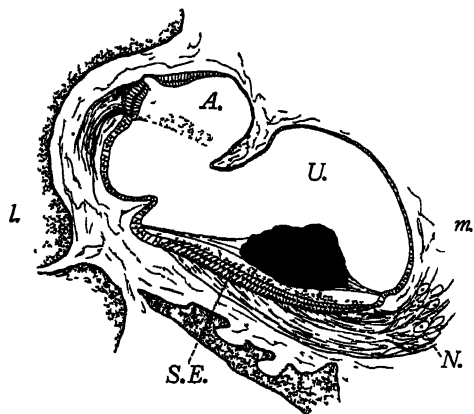


Abb. 3. Forelle.

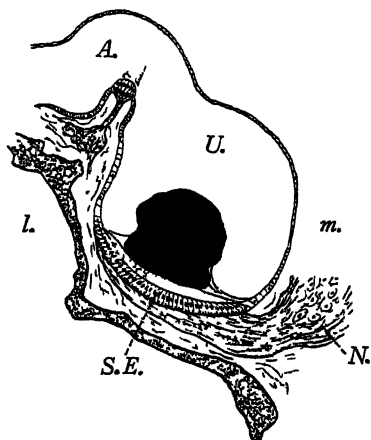


Abb. 4. Elritze.

Querschnitt durch den Utriculus (U.) entsprechend Linie I in Abb. 1 und 2. Otolith (Lapillus) schwarz. *l.* = lateral, *m.* = medial, *S.E.* = Sinnesepithel, *N.* = Nerv, *A.* = Ampulle des horizontalen Bogenganges.

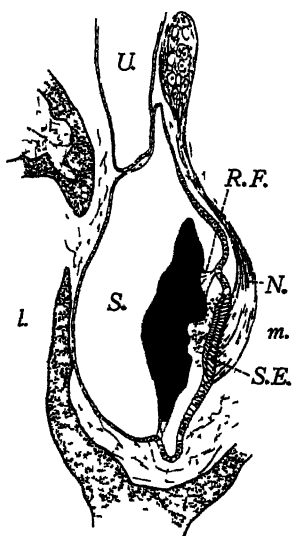


Abb. 5. Forelle.

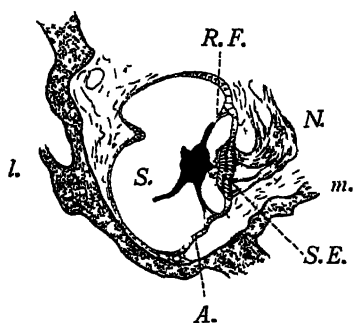


Abb. 6. Elritze.

Querschnitt durch den Saccus entsprechend Linie II in Abb. 1 und 2. Otolith (Sagitta) schwarz. *l.* = lateral, *m.* = medial, *S.E.* = Sinnesepithel, *N.* = Nerv, *U.* = Utriculus, *S.* = Saccus, *R.F.* = Randfasern, *A.* = Ausweichstelle.

noch kleiner sein als in Abb. 1; in seltenen Fällen kann sie vollständig fehlen. Die Verbindung zwischen Saccus und Lagena kann sehr eng, sie kann aber auch so weit sein, dass diese Teile äusserlich nicht voneinander abgegrenzt erscheinen.

Dasselbe gilt für die Verbindung zwischen Sacculus und Utriculus. Die Verengung der Öffnung kann hier bis zum Verschluss führen, sodass bei manchen Arten—bei Meerestischen häufiger als bei Süßwassertischen—die pars inferior des Labyrinth

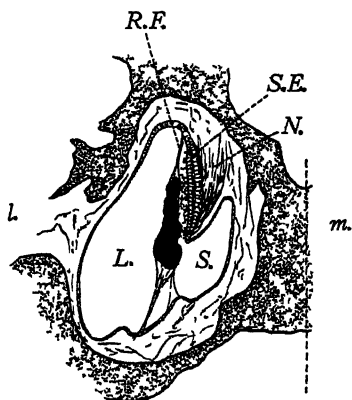


Abb. 7. Forelle.

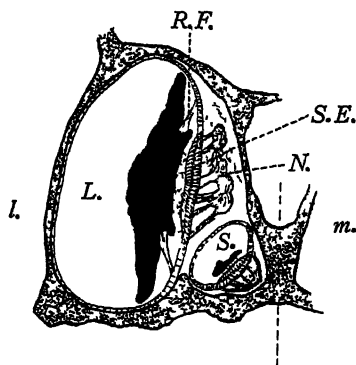


Abb. 8. Elritze.

Querschnitt durch die Lagena entsprechend Linie III in Abb. 1 und 2. Otolith (Asteriscus) schwarz. l.=lateral, m.=medial, S.E.=Sinnesepithel, N.=Nerv, R.F.=Randfasern, L.=Lagena, S.=Sacculus. Die vertikale gestrichelte Linie gibt die Medianlinie an.



Abb. 9.

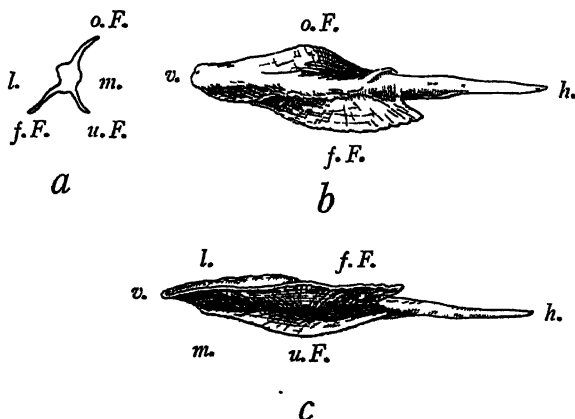


Abb. 10.

Abb. 9. Forelle. Linke Sagitta (Otolith des Sacculus) von aussen.

Abb. 10. Elritze. Linke Sagitta. (a) im Querschnitt, (b) von aussen, (c) von unten. l.=lateral, m.=medial, v.=vorne, h.=hinten, o.F. und u.F.=oberer und unterer Flügel zum Ansatz der Randfasern. f.F.=freier Flügel zum Auffangen der Schallwellen.

riths von der pars superior vollständig getrennt ist. Die papilla neglecta kann fehlen¹. Über die Bedeutung dieser Verschiedenheiten sind wir nicht unterrichtet.

(b) *Ostariophysen-Typus*. Bei den vier Knochenfischfamilien der *Ostariophysen* (Cyprinidae, Siluridae, Characinidae und Gymnotidae, vgl. S. 214) ist die pars

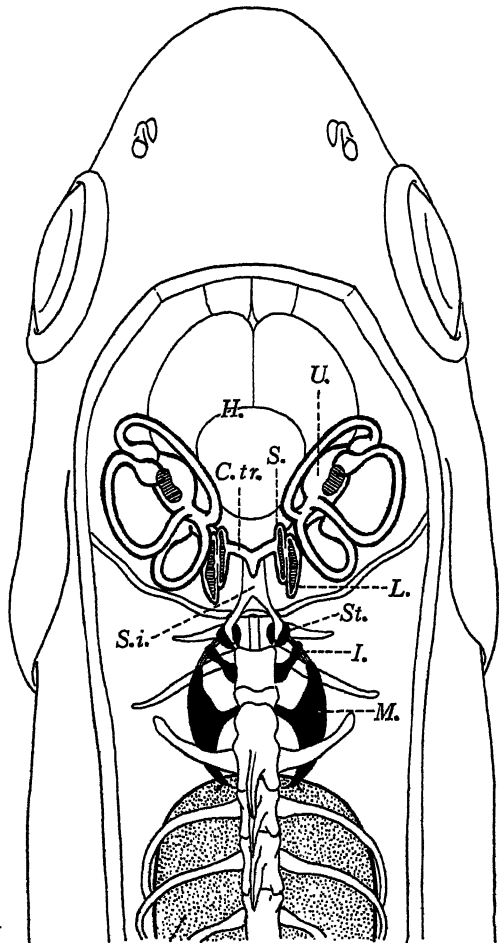
¹ Näheres über die anatomischen Verhältnisse findet man bei Retzius (1881), Bierbaum (1914), Kolmer (1927), Werner (1928), Yamanoto (1929), Wohlfahrt (1932, 1933), de Burlet (1934), v. Boutteville (1935), Denker (1935).

superior nicht abweichend gebaut, aber die pars inferior sieht ganz anders aus (Abb. 2). Der Sacculus ist langgestreckt, sein Otolith, die Sagitta, pfeilförmig und an der Oberfläche eigenartig modelliert (Abb. 6 und 10). Die lateral vom Sacculus gelegene und mit ihm durch eine enge Öffnung verbundene Lagena ist hier stets verhältnismässig gross, aber nicht von wesentlich anderer Form wie beim Normaltypus (Abb. 2 und 8).

Die besondere Gestalt des Sacculus-Otolithen der Ostariophysen (vgl. Abb. 9 und 10) ist offenbar funktionell bedingt durch die Verbindung des Sacculus mit der Schwimmblase. Diese Verbindung wird durch die "Weberschen Knöchelchen" hergestellt. Sie besteht in dieser Form bei allen Ostariophysen und nur bei diesen (Abb. 11). Die beiden Sacculi der Labyrinth stehen durch einen mit Endolymph gefüllten Canalis communicans (*C. tr.*) untereinander in Zusammenhang. Von ihm reicht ein Blindsack nach rückwärts in einen mit Perilymphe gefüllten Raum, den Sinus impar (*S. i.*). Dieser Perilymphraum führt rechts und links vom ersten Wirbel an ein Fensterchen in der Schädelkapsel, das von aussen durch ein kleines Knöchelchen (Stapes, *St.*) verschlossen ist. Stapes, Incus (*I.*) und Malleus (*M.*) bilden die Kette der Weberschen Knöchelchen, deren letztes mit der Schwimmblasenwand verwachsen ist.

Wie zuerst de Burlet (1929) für *Amiurus* und andere Siluriden, dann Wohlfahrt (1932) und Tanturri (1933) für *Phoxinus* und v. Boutteville (1935) für andere Cypriniden, Characiniden und Gymnotiden gezeigt haben, wird die Gestalt des Sacculus-Otolithen bei diesen Fischen

verständlich durch die Annahme, dass er Druckreize auffangen soll, die ihm von der Schwimmblase her durch den geschilderten Apparat zugeführt werden. Solche Druckreize, die von der Schwimmblase auf dem Wege über die Weberschen Knöchelchen, Sinus impar und Canalis transversus in den Sacculus gelangen, treffen



Sch.

Abb. 11. Die Verbindung zwischen Schwimmblase und Labyrinth durch die Weberschen Knöchelchen bei den Ostariophysen. Schwarz: die Weberschen Knöchelchen (*M.* = Malleus, *I.* = Incus, *St.* = Stapes), *Sch.* = Schwimmblase, *S. i.* = Sinus impar (Perilymphraum), *C. tr.* = Canalis transversus, *S.* = Sacculus, *L.* = Lagena, *U.* = Utriculus, *H.* = Hirn (hinterer Teil entfernt).

hier auf eine seitlich vorspringende Leiste des Sacculus-Otolithen (Abb. 12, vgl. auch Abb. 6 und 10), sodass dieser bewegt werden muss und die ihm anliegende Nervenendstelle reizen wird. Diese Auffassung wird gestützt durch den Nachweis einer sehr *dünnen* Stelle in der Sacculuswand unterhalb des Otolithen, die bei Druck leicht

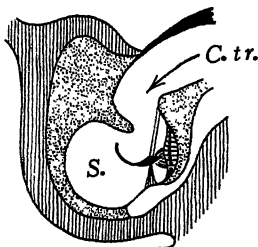


Abb. 12. Schematischer Querschnitt durch den Sacculus und Canalis transversus. Schallwellen, die von der Schwimmblase durch die Weberschen Knöchelchen und den Canalis transversus (C. tr.) dem Sacculus (S.) zugeleitet werden, werden hier durch den Flügelfortsatz des Sacculus-Otolithen aufgefangen. (Nach de Burlet.)

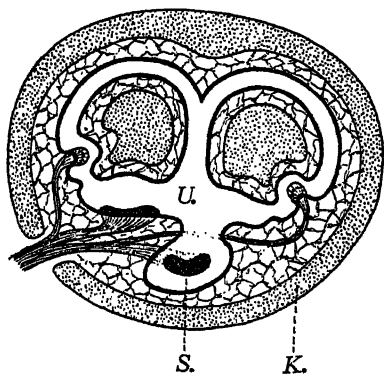


Abb. 13.

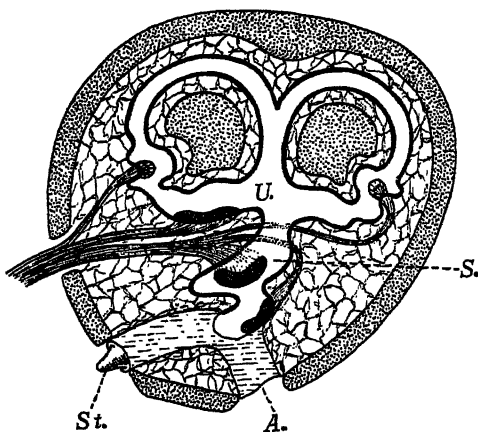


Abb. 14.

Abb. 13. Schema eines Labyrinths *ohne* perilymphatischen Zuleitungsweg für Schallwellen. Der perilymphatische Raum zwischen dem häutigen Labyrinth und der Skeletkapsel ist von Bindegewebszügen durchsetzt. U.=Utriculus, S.=Sacculus, K.=Kapsel des Labyrinths. (Nach de Burlet.)

Abb. 14. Schema eines Labyrinths *mit* perilymphatischem Zuleitungsweg für Schallwellen. Ein Teil des perilymphatischen Raumes ist frei von Bindegewebszügen (ductus perilymphaticus zwischen St. und A.). Die Schallwellen werden durch einen Stempel (St.) auf den ductus perilymphaticus übertragen und gelangen so an eine Sinnesendstelle der pars inferior. A.=Ausweichstelle, die zum Ausgleich der Druckschwankungen in der Labyrinthkapsel vorgesehen ist. U.=Utriculus, S.=Sacculus. (Nach de Burlet.)

nachgeben kann und dadurch eine Bewegung des Otolithen erst ermöglicht ("Ausweichstelle", A. in Abb. 6).

Diese Einrichtungen haben nach de Burlet (1929) den Sinn, *Schallwellen* über die Schwimmblase und die Weberschen Knöchelchen auf den Sacculus-Otolithen zu übertragen. Er hält auf Grund der anatomischen Befunde den *Sacculus der Ostariophysen* für ein schallperzipierendes Organ. Er ist der Meinung, dass ein

Labyrinth *ohne* eine Perilymphbahn als Zuleitungsweg für Schallwellen und *ohne* Ausweichstelle ein rein statisches Organ sein müsse. Diesem letzteren Typus entspricht aber das Labyrinth der Nicht-Ostariophysen¹ (vgl. de Burlet 1934, S. 1293 und 1333, und seine Schemata Abb. 13 und 14).

Hiermit gewinnt die Frage ein besonderes Interesse, ob die Nicht-Ostariophysen die Töne durch das Labyrinth wahrnehmen und ob bei den Ostariophysen tatsächlich der Sacculus das Gehörorgan ist.

(3) *Physiologische Untersuchungen über den Sitz der Tonperzeption*

An *Nicht-Ostariophysen* hat man nur wenige Untersuchungen gemacht. Nach Parker (1904) reagiert *Fundulus heteroclitus* (ein Cyprinodontide) auf Saitentöne (40 v.d.) und Stimmgabeltöne (128 v.d.) durch Flossenbewegungen. Nach beiderseitiger Durchschneidung des Nervus acusticus, also nach vollständiger Ausschaltung der Labyrinth, reagierte er auf den Saitenton nur mehr selten und schwach, auf den Stimmgabelton überhaupt nicht mehr. Den naheliegenden Einwand, dass das Ausbleiben der Reaktionen auf eine *allgemeine* Schädigung des Fisches durch den schweren Eingriff zurückzuführen sei, suchte er durch Kontrollversuche zu entkräften: nach eingreifenden Operationen anderer Art, Durchtrennung des N. trigeminus, N. facialis, N. lateralis und sogar des Rückenmarks auf der Höhe des vierten oder fünften Wirbels wurden die Töne noch durch gute Reaktionen beantwortet. Aber wenn ein strenger Kritiker meint, dass diese Operationen das allgemeine Befinden doch weniger beeinträchtigt haben als die Durchschneidung des N. acusticus und die hiermit verbundene Verletzung des Schädels, so wird er schwer zu überzeugen sein.

An *Cynoscion regalis*, einem Acanthopterygier, versuchte Parker (1910) den Sitz der Schallperzeption noch genauer festzulegen. Nach Entfernung des Utriculus und der Bogengänge blieben die Schallreaktionen erhalten. Die Entfernung des Sacculus war nicht möglich. Parker versuchte aber die Sacculus-Otolithen durch Nadeln, die vom Dach der Mundhöhle aus eingestochen wurden, vom Sinnesepithel abzudrängen und so auszuschalten. Es war dann die Reaktion auf Schallreize deutlich verschlechtert. An Haifischen (*Mustelus canis*) fand er (1910), dass nach beiderseitiger Eröffnung des Sacculus und Auswaschen der Otolithen die Schallreaktionen verschlechtert waren, und zwar in gleichem Masse, wie nach beiderseitiger Durchtrennung des N. acusticus. Auch diese Versuche sprechen—entgegen der Auffassung von de Burlet—für eine Hörfunktion des Sacculus bei den Nicht-Ostariophysen. Aber streng beweisend sind sie nicht. Es lässt sich nicht zuverlässig beurteilen, wie weit die Fische durch die Operation an sich geschädigt waren. Die Reaktionen sind nach dem Eingriff nicht ausgeblieben, sondern waren nur vermindert. Also waren entweder Sacculus und Lagena nicht restlos ausgeschaltet, oder es waren an der Schallwahrnehmung andere Organe beteiligt. Letzteres ist wahrscheinlich, weil die Schallquelle unzuweckmässig gewählt war (vgl. S. 211).

Rode (1927, 1929) meint, dass Stichlinge (*Gasterosteus*) Wasserschwingungen von geringer Frequenz (2–60 in der Sekunde) durch die Seitenorgane und durch das

¹ Mit wenigen Ausnahmen (s. S. 238, 239).

Labyrinth wahrnehmen. Seine Versuche sind aber unkritisch ausgeführt. Die Ausschaltung der Seitenorgane war unvollständig. Die Operationen am Labyrinth werden so beschrieben, dass man nicht weiss, was eigentlich zerstört wurde. Was er als Folge der Ausschaltung von Seitenorganen und Labyrinth deutet, kann sehr leicht die Folge einer allgemeinen Schädigung des Wohlbefindens sein. Es scheint mir nicht einmal erwiesen, dass die von ihm beschriebenen Reaktionen—Fluchtbewegung nur im Moment des Einschaltens seines Apparates—mit der Frequenz der Schwingungen irgend etwas zu tun hat.

Ob die Tonwahrnehmung durch das Labyrinth erfolgt, ob also ein *Gehörsinn* vorliegt, ist demnach *für Nicht-Ostariophysen bis jetzt nicht sicher entschieden*. Aber die Versuche Parkers sprechen dafür (vgl. auch S. 232).

Auch die älteren Versuche an *Ostariophysen* geben Anlass zu mancher Kritik. Als Versuchsfische wurden Goldfische (*Carassius auratus*) und Zwergwelse (*Amiurus nebulosus*) benützt.

Kreidl (1895) hat als erster feststellen wollen, ob bei Fischen das Labyrinth an der Schallperzeption beteiligt ist. Er verneint es. Er konnte an Goldfischen, deren Erregbarkeit durch Strychnin künstlich gesteigert war, Reaktionen auf heftige Schallreize (z. B. Revolverschüsse) beobachten. Da sie auch nach Extraktion der Labyrinth eintraten, schreibt er sie dem Tastsinn zu. Aber seine Operationstechnik war mangelhaft. Es ist—wie schon Bigelow (1904) erkannte—sehr wahrscheinlich, dass er nur den Utriculus und die Bogengänge entfernt hat und dass der Sacculus und die Lagena nicht zerstört wurden. Darum ist seine Schlussfolgerung nicht beweiskräftig. Mit Goldfischen haben auch zwei Schüler Parkers gearbeitet, Bigelow und Manning. Parkers Angaben für *Fundulus* konnte Bigelow (1904) am Goldfisch bestätigen: die spontanen Reaktionen auf Stimmgabeltöne (100 v.d.) bleiben aus, wenn der N. acusticus beiderseits durchschnitten ist. Da sich seine Fische von der Operation vollständig erholten und wochenlang am Leben blieben, kann man das Ausbleiben der Reaktionen hier nicht gut auf eine allgemeine Schädigung zurückführen. Recht überzeugend wird ein solcher Einwand von Bigelow selbst auch durch folgenden Versuch widerlegt: Einem Goldfisch, der besonders gut auf Töne reagierte, wurden als Vorbereitung für die spätere Durchtrennung der Nervi acustici zwei Löcher in den Schädel gebohrt, aber die Nerven selbst nicht verletzt. Dann wurden schwere Eingriffe anderer Art vorgenommen: Durchschneidung des Rückenmarkes auf der Höhe der Brustflossen, beiderseitige Durchschneidung des N. lateralis, N. trigeminus, N. facialis. Trotzdem reagierte der Fisch noch lebhaft auf Töne. Als aber nun mit einem unbedeutenden Eingriff durch die vorgebohrten Löcher die N. acustici durchtrennt wurden, war es mit den Reaktionen zu Ende. Er schliesst daraus mit Recht, dass der Ton durch das Labyrinth perzipiert wird. Um Kreidls entgegengesetzten Befund nachzuprüfen, versuchte er nach dessen Methode das ganze Labyrinth zu extrahieren. Dabei konnte er aber nur den Utriculus herausziehen, Sacculus und Lagena blieben im Schädel zurück. Diese Fische *reagierten* noch. Daraus folgt, dass der von Bigelow angewandte Stimmgabelton (100 v.d.) durch die pars inferior des Labyrinths

wahrgenommen wird. Manning (1924) benutzte einen Apparat, mit dem er Schwingungen von 43, 86, 172, 344, 688, 1376 oder 2752 v.d. auf das Wasser übertragen konnte. Um die Bedeutung der Labyrinthteile für die Tonwahrnehmung kennen zu lernen, prüfte er Fische, denen das Labyrinth teilweise zerstört war. Die Extraktion des Utriculus und der Ampullen bot keine Schwierigkeit. Die Entfernung des Sacculus und der Lagena gelang ihm nicht. Darum wurden nur ihre Otolithen mit einer durch den Schädel gestochenen Nadel zerbrochen. Sein Ergebnis war, dass hohe Töne (2752 und 1376 v.d.) nur durch Sacculus und Lagena wahrgenommen werden, mittelhohe Töne nur (688 und 344 v.d.) oder teilweise (172 und 86 v.d.) durch den Utriculus, tiefe Töne (86 und 43 v.d.) aber durch Organe der Haut. Mannings Angaben stimmen nicht ganz zu den älteren Befunden von Parker und Bigelow (vgl. v. Frisch u. Stetter 1932, S. 691, 692). Es hat sich auch später nicht bestätigt, dass der Utriculus an der Tonwahrnehmung beteiligt ist.

An zwei Zwergwelsen (*Amiurus nebulosus*), die sehr deutlich auf Pfeifen reagierten, hat schon Haempel (1911) die Labyrinthzelle zu entfernen versucht. Einer von den beiden Welsen gab auch nachher noch Reaktionen. Bei seiner Untersuchung zeigte sich, dass Sacculus und Lagena erhalten geblieben waren. Der andere reagierte nicht mehr. Bei ihm waren die Labyrinthzellen vollständig exstirpiert. Die Schlussfolgerung, dass die Pfeifentöne durch Sacculus und Lagena gehört wurden, war nicht zwingend. Denn der Fisch, bei dem die Operation vollständig gelungen war, lag nachher schwer geschädigt an der Wasseroberfläche, während der andere noch herumschwamm. Das Ausbleiben der Reaktionen konnte der allgemeinen Schädigung zugeschrieben werden. Durch viel umfangreichere Experimente haben Parker und van Heusen (1917) das Problem am Zwergwels verfolgt. Sie wollten sehen, in welchem Masse Hauttastsinn, Seitenorgane und Labyrinth an der Perception mechanischer Reize beteiligt sind. Sie haben deshalb die Reaktionen von Welsen untersucht, bei welchen einer, zwei oder alle drei von den genannten Sinnen ausser Funktion gesetzt waren. Uns interessiert hier besonders, dass die Welse mit normalem Labyrinth auf einen Pfeifenton 100 % positive Reaktionen gaben, nach Ausschaltung des Labyrinths aber 0 % positive Reaktionen. Dies scheint zu beweisen, dass der Pfeifenton durch das Labyrinth wahrgenommen wird. Aber die Autoren sagen in ihrer Arbeit (S. 471), dass die normalen Fische auf den Pfeifenton in der Regel überhaupt nicht reagierten, wenn sie sich *unter* der Wasseroberfläche aufhielten; die verzeichneten positiven Reaktionen wurden von Fischen erhalten, die direkt *an der Wasseroberfläche* waren. Später (S. 486) erwähnen sie, dass die Tiere nach Ausschaltung des Labyrinths meist am Boden liegen; wenn sie schwimmen, geschieht es in rollenden oder spiraligen Bewegungen. Daraus geht offenbar hervor, dass sie sich nach der Labyrinthoperation nicht mehr an der Oberfläche aufhielten und daher nicht mehr unter jenen Bedingungen geprüft werden konnten, unter denen allein zuverlässige Reaktionen zu erwarten waren. Darum sind auch diese Versuche kein strenger Beweis für eine Tonperzeption durch das Labyrinth.

Die bisher besprochenen Experimente lassen aber doch vermuten, dass das Labyrinth, und zwar die pars inferior (Sacculus und Lagena), der hauptsächliche Sitz der Tonwahrnehmung ist. Dass alle diese Versuche nicht überzeugend gewirkt haben, liegt an der Operationsweise der Autoren und an der Reaktionsweise

der Fische: Die Methode der Operationen war unbefriedigend, der Erfolg nicht hinreichend gesichert und kontrolliert. Die beobachteten Ausfallerscheinungen bezogen sich stets auf *Spontanreaktionen* der Fische, die nicht genügend deutlich und zuverlässig sind.

Wir (v. Frisch und Stetter 1932) haben an Elritzen (*Phoxinus laevis*) versucht, die Frage mit einer *verbesserten Operationstechnik* zu lösen und wir haben uns der *Dressurmethode* bedient, weil man mit ihr bei normalen Elritzen so ausgezeichnete und klare Reaktionen erhält. Unsere Tiere waren alle durch Exstirpation beider Augen geblendet, sodass optische Fehlerquellen ausgeschlossen waren. Jeder Fisch wurde nach Abschluss der Versuchsreihe konserviert und der Erfolg der Operation an vollständigen Schnittserien durch die Labyrinthregion des Schädels nachgeprüft.

Wir haben die Bedeutung der *pars superior* und der *pars inferior* des Labyrinths getrennt untersucht.

Die *Entfernung der pars superior* ist nicht schwer. Wir bohren jederseits mit einem feinen Zahnbohrer ein kleines Loch in das Schädeldach und holen mit einem feinen Häkchen den Utriculus samt seinem Otolithen und die Bogengangampullen heraus¹. So operierte Tiere haben ihren *Sinn für die Orientierung im Raume vollständig verloren*. Meist liegen sie am Boden des Aquariums, oft auf der Seite oder mit dem Rücken nach unten. Wenn sie aufschwimmen, taumeln sie durch das Wasser. Aber sie *reagieren auf Töne* noch ebenso gut wie normale Fische. Wir haben 15 Elritzen nach beiderseitiger Entfernung des Utriculus auf Töne dressiert. Eine von ihnen starb bald nach der Operation, bei allen anderen war die Dressur erfolgreich. Sie lernten ebenso schnell wie normale Elritzen, auf den Futterton zu reagieren. Sie lernten auch ebenso wie normale Fische, einen Futterton von einem Warnton zu unterscheiden. Wir haben sie durch den ganzen Tonbereich geprüft und die Hörgrenzen normal gefunden. Sie beantworteten auch noch sehr leise Töne². *Die pars superior des Labyrinths ist also offenbar am Hörvermögen der Elritze nicht beteiligt.*

Die *Entfernung der pars inferior* (also des Sacculus und der Lagna, s. Abb. 2, S. 221) ist schwierig. Aber sie ist möglich auf dem Wege durch die Kiemenspalte. Wir narkotisieren die Elritze mit Urethan, versorgen sie durch einen Mundschlauch mit Atemwasser, halten durch Fadenzüge den Kiemendeckel und die Muskulatur bei Seite (Abb. 15), drängen mit einem stumpfen Instrument die Kiemenbogen nach unten, bohren unter dem binokularen Mikroskop ein kleines Loch in die seitliche Schädelwand und holen Sacculus und Lagna samt ihren Otolithen mit dem Häkchen heraus. So operierte Elritzen zeigen, wenn der Utriculus unbeschädigt geblieben ist, nicht die geringste Gleichgewichtsstörung. Daraus folgt, dass *Sacculus und Lagna mit ihren Otolithen für die Orientierung im Raume ohne Bedeutung sind*. Löwenstein (1932) hat sich mit dieser Frage noch besonders befasst

¹ Die Ampulle des hinteren vertikalen Bogenganges bleibt hierbei im Schädel zurück. Sie kann durch ein besonderes Bohrloch entfernt werden. An dem Verhalten der Fische gegenüber Schallreizen ändert dies nichts.

² Die Hörschärfe kann gegenüber normalen Elritzen etwas vermindert sein. Dies findet aber darin eine Erklärung, dass bei blinden Elritzen nach Exstirpation des Utriculus das Volumen der Schwimmblase abnimmt (v. Frisch, 1934). Vgl. den folgenden Abschnitt über die Bedeutung der Schwimmblase für die Hörschärfe.

und zeigen können, dass alle bei einem geblendeten Fisch mit normalem Labyrinth nachweisbaren statisch-dynamischen Reflexe vom Utriculus und den Bogengängen ausgehen. Nach Ausschaltung der pars inferior bleiben diese Reflexe alle erhalten, nach Ausschaltung der pars superior sind sie alle vernichtet.

Wir haben 13 Elritzen¹ nach beiderseitiger Entfernung des Sacculus und der Lagena auf Töne zu dressieren versucht. Bei normalen Elritzen liegt die obere Hörgrenze zwischen 5000 und 6000 v.d. (etwa e^5 – g^5). Nach Exstirpation der pars inferior haben wir mit Tönen höher als 145 v.d. (d) trotz langer Bemühungen nie mehr eine Reaktion erzielt. Diese Fische reagieren nur noch auf tiefe Töne, zuverlässig auf solche tiefer als 100 v.d. (G), und nur dann, wenn die tiefen Töne sehr laut sind. *Die Töne in mittleren und hohen Lagen* (von 100–150 v.d. bis aufwärts zu 5–6000 v.d.) *werden also ausschliesslich durch die pars inferior—Sacculus und Lagena—wahrgenom-*

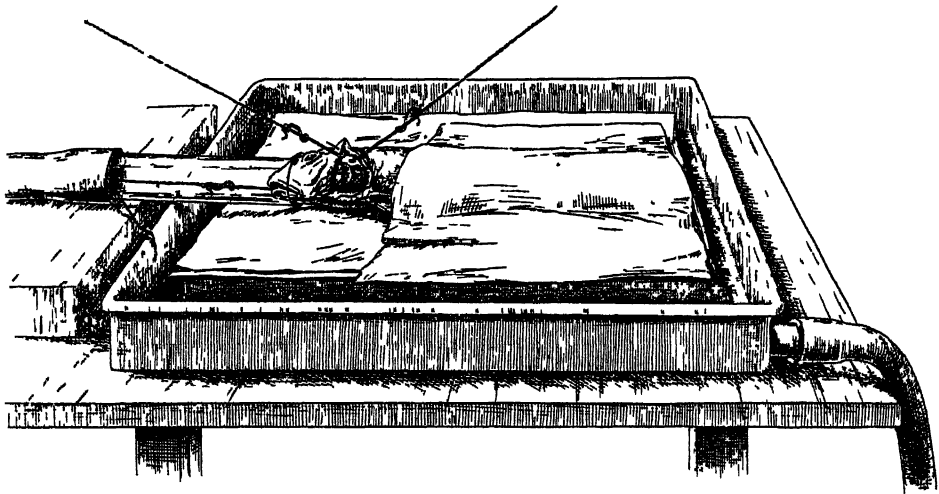


Abb. 15. Lagerung des Fisches zur Operation der pars inferior des Labyrinths.

men. Nach der auf S. 220 gegebenen Definition bezeichnen wir diese Tonwahrnehmung durch das Labyrinth als *Gehör*.

Man könnte einwenden, das Misslingen der Tondressur sei nicht der Entfernung des Sacculus und der Lagena, sondern einer allgemeinen Schädigung der Fische durch die Operation zuzuschreiben. Dieser Einwand wird durch folgende Erfahrungen widerlegt: (1) Die Elritzen waren in ihrem Allgemeinzustand nicht merklich geschädigt; wir haben sie nach der Operation durchschnittlich noch 4–5 Monate (eine $1\frac{1}{2}$ Jahre) munter und fresslustig in Dressur gehabt. (2) Die Exstirpation der *pars superior* schädigt sie in höherem Grade, wie aus dem Verhalten der Fische und aus ihrer erheblich kürzeren Lebensdauer hervorgeht; trotzdem lassen sich *diese* Elritzen noch gut auf Töne dressieren. (3) Nach einseitiger Entfernung der pars inferior reagieren sie wie normale Tiere. Entfernt man nach einigen Wochen auch die pars inferior der anderen Seite, so sind die Reaktionen auf die mittelhohen und hohen Töne vernichtet, obwohl der zweite Eingriff nicht schwerer war als der erste. (4) Nach beiderseitiger Exstirpation der pars inferior lassen sich die Elritzen

¹ Und später noch viel mehr, mit demselben Ergebnis.

auf tiefe, laute Töne noch dressieren; auch geben sie bei Erregung ihrer chemischen Sinnesorgane und der Seitenorgane bei Annäherung des Futters prächtige Reaktionen. Es ist also durch den Verlust der pars inferior nicht ihre allgemeine Reaktionsfähigkeit geschädigt, sondern ihr Hörvermögen vernichtet worden.

Auf Töne mit einer Schwingungszahl von weniger als 100–150 v.d. reagieren die Elritzen noch, nachdem die pars inferior beiderseits entfernt ist. Wir konnten aber feststellen, dass bei *normalen* Fischen die pars inferior des Labyrinths bis herunter zu einer Frequenz von 16–32 v.d. an der Tonwahrnehmung *beteiligt* ist. Hier liegt die *untere Hörgrenze* für das Labyrinth der Elritze. Wir haben dies so nachgewiesen, dass wir die Fische in normalem Zustand, dann nach einseitiger und schliesslich nach doppelseitiger Exstirpation der pars inferior mit tiefen Tönen von abgestufter Intensität geprüft haben. Durch *einseitige* Entfernung der pars inferior wurde die Reizschwelle nicht nennenswert beeinflusst. Nach Operation der zweiten Seite, also nach vollständiger Ausschaltung von Sacculus und Lagenä, hat sich für die Töne C (65 v.d.) und C₁ (32 v.d.) übereinstimmend eine wesentliche Erhöhung der Reizschwelle ergeben. Gemessen an der Energie der Schwingungen, war die Empfindlichkeit der Tiere für diese Töne auf etwa 1/250 ihrer normalen Hörschärfe gesunken. Für den Ton C₂ (16 v.d.) hat sich aber auch nach beiderseitiger Operation die Reizschwelle nicht wesentlich verändert. An der Wahrnehmung dieses tiefsten Tones ist also das Labyrinth nicht mehr merklich beteiligt.

Man wird nun fragen, durch welches Sinnesorgan die Fische diese tiefen Töne wahrnehmen, wenn das Labyrinth ausgeschaltet ist. Verschiedene Autoren haben eine Beteiligung des Seitenorgansystems an der Tonperzeption vermutet und zum Teil auch experimentell zu begründen versucht. Die Experimente sind aber nicht beweiskräftig¹. Wir konnten zeigen, dass jener Rest von Wahrnehmungsvermögen für tiefe Töne, der nach Entfernung der pars inferior noch besteht, auch nach vollständiger Ausschaltung des Seitenorgansystems erhalten bleibt. Die Seitenorgane haben also mit der Tonwahrnehmung nichts zu tun². Da wir weiter auch eine Beteiligung des Utriculus ausschliessen konnten, kommen wir per exclusionem zu der Annahme, *dass die tiefen Töne von der Elritze durch den Tastsinn der Haut perzipiert werden*—wie auch wir selbst tiefe Töne bei genügender Intensität durch die Haut fühlen können. So wie bei uns, überschneiden sich auch bei der Elritze in einem gewissen Gebiet die Funktionsbereiche von Tastsinn und Gehörsinn. Wir können unsere Ergebnisse über diesen Punkt in folgender Übersicht zusammenfassen:

Ton	Untere Hörgrenze 16–32 v.d.			Wahrgenommen durch die pars inferior des Labyrinths der Elritze						Obere Hörgrenze 5000–6000 v.d.	
	C ₂	C ₁	C	c	c ¹	c ²	c ³	c ⁴	c ⁵	c ⁶	
Frequenz in v.d.	16	32	65	129	259	517	1035	2069	4138	8277	
	Wahrgenommen durch den Tastsinn der Haut der Elritze										

¹ Vgl. v. Frisch u. Stetter (1932), S. 772.

² Über ihre Funktion bei der Wahrnehmung von Wasserströmungen vgl. Dykgraaf (1933). Über die Technik ihrer Ausschaltung vgl. auch v. Frisch u. Stetter (1932), S. 773.

Aus den bisher erwähnten Versuchen geht nicht hervor, welchen Anteil der Sacculus und welchen Anteil die Lagena an der Tonwahrnehmung hat. Nach den Anschauungen von de Burllet (s. S. 225) hätte ja nur der Sacculus den Bau eines Gehörorgans, die Lagena dagegen den Bau eines statischen Organs. Wir wissen aber, dass ihr keine statische Funktion zukommt. Schon dadurch wird wahrscheinlich, dass auch sie Hörfunktion hat. Hierfür spricht auch ein Befund von D. A. Ross (zitiert nach Tait 1932)¹, der an einem Cypriniden (*Catostomus*) mit einem Galvanometer nach der Methode Pipers (s. unten) Stromschwankungen am Labyrinth bei Schallreizung nachweisen konnte, die nach Entfernung der Sacculus-Macula schwächer wurden, aber nicht ausblieben. Um den vollen elektrischen Effekt zu geben, war auch die Lagena notwendig. Ich selbst habe versucht, den relativen Anteil des Sacculus und der Lagena am Hörvermögen durch ihre getrennte Ausschaltung zu klären. Diese Arbeit ist aber noch nicht abgeschlossen.

Ich komme jetzt noch einmal auf die *Frage des Hörvermögens bei Nicht-Ostariophysen* zurück. Nachdem wir wissen, dass die Elritze durch die pars inferior des Labyrinths hört, haben wir kaum eine Ursache, den alten Angaben Parkers so misstrauisch gegenüberzustehen, wie es vielfach geschieht. Er hat versucht zu zeigen, dass *Fundulus heteroclitus* durch das Labyrinth, *Cynoscion regalis* im Besonderen durch den Sacculus Töne wahrnimmt (vgl. S. 226). Als weiteres Argument für eine Schallperzeption durch die pars inferior bei Nicht-Ostariophysen lassen sich Pipers Versuche am Hecht (*Esox lucius*, 1907, 1910) ins Feld führen. Er konnte an überlebenden Hechtköpfen durch Ableitung von der Gegend des Sacculus-Otolithen, nicht aber von anderen, nahe benachbarten Stellen, mit einem empfindlichen Galvanometer Stromschwankungen bei Schallreizung nachweisen. Die Schallreizung erfolgte meist durch Membranpfeifen, deren Grundtöne etwa 100 und 260 v.d. hatten. Es genügten schwache Töne, auch leises Klopfen an die Glaswände des Aquariums in welchem der Hechtkopf untergebracht war, um die Stromschwankungen zu erzeugen, nicht aber schallose, mechanische Erschütterungen des Präparates, Umrühren des Wassers und dergleichen. Pipers Versuche sind neuerdings von D. A. Ross (zitiert nach Tait, 1932) bestätigt worden. Er hat Pfeifentöne von 50–130 v.d. angewendet. Über Pipers Ergebnis hinaus führt seine Beobachtung, dass beim Hecht nach Entfernung des Sacculus-Otolithen und nach Zerstörung der Sacculus-Macula die Stromschwankungen ausbleiben—wobei unsicher ist, ob die kleine Lagena mit zerstört wurde².

Nach den Anschauungen von de Burllet (S. 225) hat bei den Nicht-Ostariophysen das ganze Labyrinth den Bau eines statischen Organs. Aber verschiedene Erfahrungen haben gelehrt (Parker, 1910; Maxwell, 1923; Werner, 1929), dass auch bei ihnen (wie wir bei *Phoxinus* gefunden haben) die Zerstörung der pars inferior keine

¹ Eine ausführliche Veröffentlichung der Versuche von Ross ist nach brieflicher Mitteilung von Prof. Tait nicht erfolgt.

² Evans (1932) hat auf die gute Entwicklung der "Hörzentren" im Gehirn mancher Cypriniden, Clupeiden und Trigliden hingewiesen. Für unsere Frage wird man sich auf diese morphologischen Befunde nur mit Vorsicht stützen können. Es scheint mir nicht genügend geklärt, welchen Anteil die statisch-dynamischen Labyrinthfunktionen an der Ausbildung dieser Zentren haben.

schädlichen Folgen für das Gleichgewicht hat. Bedenkt man ferner die eben erwähnten Versuche, so ist es als wahrscheinlich zu bezeichnen, dass auch bei den Nicht-Ostariophysen die pars inferior des Labyrinths als Gehörorgan dient. Neue Untersuchungen darüber haben wir in unserem Institut begonnen.

III. DIE BEDEUTUNG DER SCHWIMMBLASE FÜR DAS HÖRVERMÖGEN

Dass bei den Ostariophysen die Schwimmblase mit der pars inferior des Labyrinths durch die Weberschen Knöchelchen verbunden ist, wurde schon mehrmals erwähnt (Abb. 11, S. 224). Weber selbst hat (1820) die von ihm entdeckten Knöchelchen mit Hammer, Amboss und Steigbügel im Mittelohr der Säugetiere verglichen und ihnen eine analoge Aufgabe zugeschrieben. Er nahm an, dass sie die Schallwellen von der Schwimmblase auf das Gehörorgan übertragen. Sörensen (1894–5) hat gezeigt, dass sie für diese Aufgabe tatsächlich geeignet sind. Wenn er an einem frisch getöteten Karpfen die Schwimmblase zum Vibrieren brachte, gerieten die Weberschen Knöchelchen in longitudinale Schwingungen, die mit der Lupe deutlich erkennbar waren. Dass unter natürlichen Verhältnissen die Schwimmblase durch Schallwellen zum Vibrieren gebracht wird, muss angenommen werden (s. S. 238) und wird durch ihre pralle Füllung mit Gas und durch die Elastizität ihrer Wandung—beides charakteristische Merkmale der Ostariophysen-Schwimmblase—noch begünstigt. An ihrer Verbindungsstelle mit den Weberschen Knöchelchen beschrieb Evans (1925, 1930) besondere Differenzierungen, welche geeignet erscheinen, die Übertragung der Vibrationen zu erleichtern, und de Burlet (1929) machte uns schliesslich mit dem schönen Auffangeapparat für die ankommenden Schallwellen im Sacculus von *Amiurus* bekannt, eine Einrichtung, die nach den Untersuchungen von Wohlfahrt (1932) und v. Boutteville (1935) bei allen Ostariophysen ausgebildet ist (vgl. S. 224).

Nach einer zweiten Ansicht sollen durch die Weberschen Knöchelchen die Änderungen des Schwimmblasenvolumens, die beim Übergang in andere Wassertiefen eintreten, dem Labyrinth angezeigt werden. Dieses soll die Aufgabe haben, die Reflexe auszulösen, durch die entweder der Gasgehalt der Schwimmblase reguliert oder der Fisch in die Wassertiefe zurückgeführt wird, an welche er durch den Füllungszustand seiner Schwimmblase angepasst ist (vgl. Hasse, 1873, S. 471; Bridge und Haddon, 1889, 1893; Baglioni, 1908; Thilo, 1908; Fiebiger, 1924; v. Kokas, 1932; Schiffers, 1934). Für diese Auffassung liegt bis heute kein überzeugender Beweis vor. *Gegen* sie sprechen die Verhältnisse bei manchen Siluriden und Cypriniden, die sich am Boden aufhalten. Die Schwimmblase ist bei ihnen sehr klein und ohne hydrostatische Bedeutung. Aber gerade der *vordere* Teil der Blase und ihre Verbindung mit den Weberschen Knöchelchen bleibt bestehen. Diese Einrichtung ist hier als Regulator des Gasgehaltes sinnlos, als Apparat mit akustischer Funktion aber ebenso verständlich wie bei frei schwebenden Formen.

Wir fragen jetzt, ob sich eine akustische Bedeutung der Schwimmblase bei den Ostariophysen experimentell nachweisen lässt.

Um dies zu prüfen, haben wir (v. Frisch u. Stetter 1932) bei Elritzen (*Phoxinus laevis*) die Schwimmblase entfernt. Wir haben diese Fische nach der Operation

viele Monate, einige von ihnen über 1 Jahr am Leben erhalten und beobachtet. Sie liessen sich gut auf Stimmgabel- und Pfeifentöne dressieren. Überraschend war, dass sie schon wenige Tage nach der Entfernung der Schwimmblase wie normale Tiere im Wasser schwebten. Sie ersetzten den Verlust der Schwimmblase dadurch, dass sie an der Wasseroberfläche Luft schnappten und so den vorderen Teil ihres Darmes mit Gasblasen füllten, bis sie richtig äquilibriert waren. Obwohl diese Luftblasen natürlich nicht mit den Weberschen Knöchelchen in Verbindung stehen, haben wir doch, um jeden Einwand nach dieser Richtung auszuschliessen, andere Elritzen gleich nach der Exstirpation der Schwimmblase in Aquarien gesetzt, in denen unter der Wasseroberfläche ein Gitter angebracht war. Diese Fische konnten also nicht an die Oberfläche, um Luft zu schlucken. Tatsächlich waren in ihrem Körper keine Gasblasen enthalten. Trotzdem war auch bei ihnen die Tondressur erfolgreich. Aus diesen Versuchen geht hervor, *dass die Elritzen auch ohne Schwimmblase hören können.*

Wir haben aber durch Anwendung abgeschwächter Pfeifentöne gefunden, dass die Elritzen nach dem Verlust der Schwimmblase *nicht mehr so gut* hören wie vorher. Die Töne müssen mit bedeutend grösserer Intensität geboten werden, damit eine Reaktion erfolgt. Dies gilt sowohl für hohe Töne (geprüft für e^4 , 2607 v.d.) wie für den mittleren Tonbereich (e^3 , 652 v.d.) und wurde neuerdings von Wolf (noch unveröffentlichte Arbeit) auch für tiefe Töne ($C-c^1$, 65–257 v.d.) bestätigt. Wolf hat in quantitativen Versuchen gefunden, dass nach Ausschaltung der Schwimmblase die Hörschärfe—gemessen an der Energie der Schwingungen—um das 40–70fache vermindert ist. *Wir sehen also in der Schwimmblasen-Labyrinth-Verbindung der Ostariophysen eine Einrichtung zur Steigerung der Hörschärfe.*

Wenn dies richtig ist, sollten *erstens* alle Ostariophysen durch eine grosse Hörschärfe ausgezeichnet sein, und es sollten *zweitens* die Nicht-Ostariophysen, die ja keine Weberschen Knöchelchen haben, allgemein schlechter auf Töne reagieren. Ersteres ist experimentell gut begründet durch Hörschärfeprüfungen an allen Familien der Ostariophysen (vgl. S. 219, 220). Für die zweite Annahme lassen sich eine Reihe von Wahrscheinlichkeitsgründen anführen:

Spontane Reaktionen auf Schallreize sind bisher fast nur bei Ostariophysen beobachtet worden (bei den Fischarten, die in der Übersicht S. 214, 215 durch ein Sternchen gekennzeichnet sind).

Reaktionen auf Schallreize, die *durch die Luft*, und daher mit verhältnismässig *geringer Intensität* auf das Fischbecken übertragen wurden, sind überwiegend bei Ostariophysen festgestellt worden (bei den Fischarten, die in der Übersicht S. 214, 215 *kursiv* gedruckt sind).

Versuche unter gleichen Bedingungen haben an Ostariophysen positive, an Nicht-Ostariophysen schlechtere oder negative Resultate gezeigt. So ist der oft zitierte, aber nie in seiner Bedeutung erkannte Widerspruch zwischen Zenneck (1903) und Bernoulli (1910) zu verstehen. Beide haben eine Glocke unter Wasser tönen lassen. Nach Zenneck ergreifen die Fische in einem Umkreis von mehreren Metern die Flucht, nach Bernoulli aber reagieren sie nicht einmal in einem Abstand von wenigen Dezimetern. Zenneck hat Ostariophysen beobachtet (*Leuciscus rutilus*, *L. dobula*,

Alburnus lucidus), Bernoulli aber Nicht-Ostariophysen (*Salmo fario*, *Lucioperca sandra*, *Anguilla vulgaris*). Stetter (1929) hat bei seinen Tondressuren an Ostariophysen ausgezeichnete Resultate gehabt (*Phoxinus laevis*, *Idus melanotus*, *Cobitis barbatula*, *Amiurus nebulosus*). Unsicher waren die Ergebnisse nur bei *Cottus gobio*, dem einzigen von ihm geprüften Nicht-Ostariophysen. Denker (1931) erzielte an Ostariophysen (*Carassius auratus*, *Idus melanotus*, *Cobitis barbatula*, *Amiurus nebulosus*) gute Reaktionen auf Töne, bei Nicht-Ostariophysen (*Salmo fario*, *Perca fluviatilis*) hatte er keinen sicheren Erfolg. *Phoxinus laevis* (Ostariophyse) reagiert sehr gut auch auf leise Schallreize, *Umbra pygmaea* (Nicht-Ostariophyse) aber nur auf sehr intensive Töne (v. Frisch und Stetter, 1932, S. 795–6).

Vielleicht wird durch weitere Untersuchungen auch noch für manche andere Fischfamilien eine ähnliche Hörschärfe nachgewiesen werden, wie für die Ostariophysen. Dann wird man wahrscheinlich bei ihnen statt der Weberschen Knöchelchen eine andere Einrichtung zur Steigerung der Hörschärfe finden. Anatomische Angaben, die diese Vermutung nahe legen, werden wir noch kennen lernen (S. 238, 239).

IV. DAS HÖREN OHNE SCHNECKE UND OHNE BASILARMEMBRAN

Die Schnecke der Säugetiere ist eine Differenzierung der pars inferior, die schon am Labyrinth der Ostariophysen—und wahrscheinlich aller Fische—der Sitz des Gehörsinnes ist. Die vergleichende Anatomie der Wirbeltiere zeigt uns die schrittweise Entwicklung der Schnecke. Den Fischen fehlt sie völlig. Die pars inferior besteht hier nur aus Sacculus und Lagena (vgl. S. 221 und Abb. 16a). In primitiver Ausbildung bei Amphibien, in typischer Entwicklung bei Reptilien tritt unmittelbar neben der Lagena eine neue Ausbuchtung mit neuer Nervenendstelle an der pars inferior auf, die papilla basilaris mit der Basilarmembran (Abb. 16b). Bei manchen Reptilien und bei den Vögeln streckt sie sich in die Länge; die Lagena wird von dem auswachsenden Gebilde mitgenommen und liegt dann an seinem distalen Ende (Abb. 16c). Bei den Säugetieren wird die papilla basilaris schneckenförmig gewunden, die Lagena verschwindet (Abb. 16d).

Die Helmholtzsche Resonanztheorie sieht in den Fasern der Basilarmembran ein System von abgestimmten Saiten und führt die Fähigkeit der Klanganalyse und der Tonunterscheidung auf diese Differenzierung zurück. Unter dem Eindruck dieser einleuchtenden Hypothese hat man die Schnecke der Säugetiere und die ihr entsprechende papilla basilaris anderer Wirbeltiere *allein* als Gehörorgan betrachtet. Die Nervenendstellen des Sacculus, der Lagena und des Utriculus hielt man für statische Organe. Für diese Auffassung schien die Anwesenheit der "Statolithen" zu sprechen, die bei den Knochenfischen als grosse, feste Kalksteinchen, bei den anderen Wirbeltieren in Gestalt zahlreicher kleiner Kalkkonkremente, diesen Nervenendstellen auf- oder angelagert sind (Abb. 16a–d).

Die Versuche an Fischen haben aber gezeigt, dass bei ihnen (mit Sicherheit bei der Elritze) die pars inferior als Gehörorgan dient. Die Nervenendstellen des Sacculus und wahrscheinlich auch der Lagena vermitteln hier die Schallwahrneh-

mung und Tonunterscheidung, obwohl sie mit Otolithen ausgestattet sind und obwohl eine Basilar membran fehlt. Es liegt nahe, an diese Tatsache einige theoretische Betrachtungen zu knüpfen.

(1) Wenn die pars inferior des Fischlabirynths ein Gehörorgan ist, so haben wir keinen Grund, dem Sacculus und—wo sie vorhanden ist—der Lagena im Labyrinth der höheren Wirbeltiere eine Hörfunktion abzusprechen. Eine solche ist

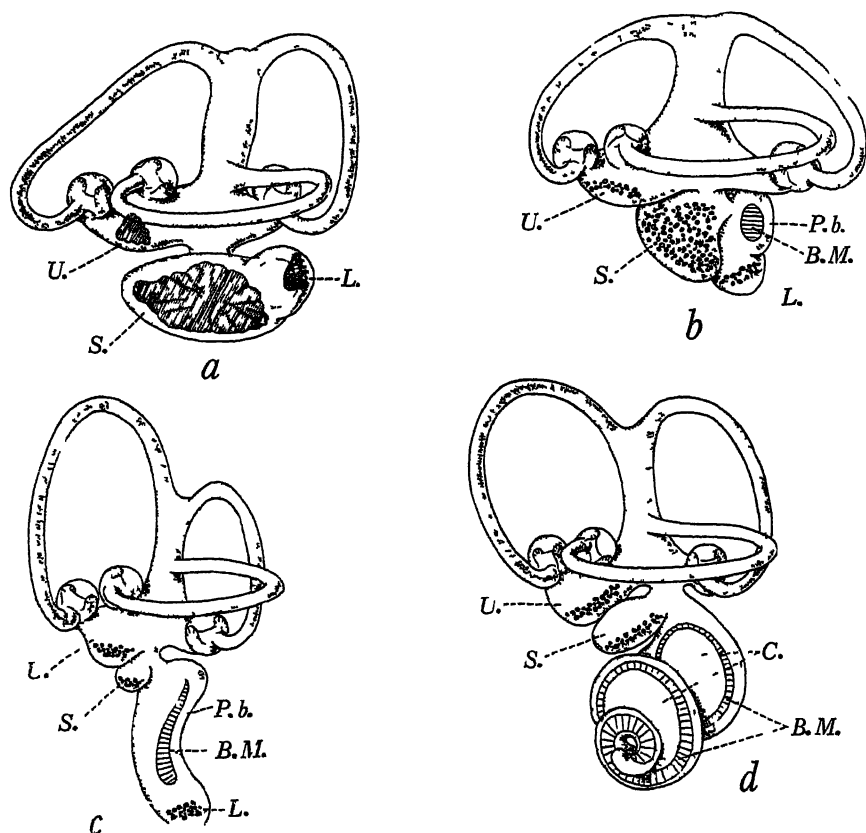


Abb. 16. Labyrinth verschiedener Wirbeltiere: (a) Fisch (Normal-Typus), (b) Schildkröte, (c) Vogel, (d) Säugetier. U.=Utriculus, S.=Sacculus, L.=Lagena, P.b.=Papilla basilaris, B.M.=Basilar membran, C.=Cochlea (Schnecke).

zwar für sie bei höheren Wirbeltieren nicht sicher nachgewiesen. Aber es häufen sich die Angaben, dass die pars inferior ganz allgemein *keine statische Funktion* hat.

Dies gilt bei den *Fischen* nicht nur für Ostariophysen (Manning, 1924; v. Frisch u. Stetter, 1932; Löwenstein, 1932), sondern auch für Nicht-Ostariophysen: für *Mustelus* (Parker, 1910; Maxwell, 1923), *Cynoscion* (Parker, 1910), *Gobius* (Werner, 1929). Auch bei *Amphibien* gehen offenbar alle statisch-dynamischen Reflexe von der pars superior aus. Laudenbach hat schon 1899 mitgeteilt, dass beim Axolotl und beim Frosch die Verletzung des Sacculus und die Entfernung seiner Otolithenmasse nicht die geringste Gleichgewichtsstörung bewirkt. Durch ein-

gehende Experimente am Frosch haben dies neuerdings McNally und Tait (1925–6) und Huddleston (1928) bestätigt. Ashcroft und Hallpike (1934) haben überdies gezeigt, dass sich vom Sacculus des Frosches auf Drehen und Neigen des Körpers keine Aktionsströme ableiten lassen, wohl aber bei akustischer Reizung (Fussstampfen, Stimmgabeltöne). Für *Reptilien* liegt eine Angabe von Tait (1932, S. 682) vor, dass bei der Klapperschlange nach Entfernung des Sacculus das Gleichgewicht nicht merklich gestört ist. Bei *Vögeln* (Tauben) fanden Huizinga und Benjamins (1928) nach Exstirpation der pars inferior nur die eine Erscheinung, dass die Raddrehung des Auges verändert war, aber in einer späteren Arbeit führt Benjamins (1934) auch diese Störung auf eine unbeabsichtigte Schädigung der pars superior zurück und kommt zu dem Ergebnis, dass auch hier die pars inferior an den statisch-dynamischen Labyrinthreflexen nicht beteiligt ist. Dieselbe Überzeugung hat man neuerdings—im Gegensatz zu den älteren Anschauungen von Magnus (1924)—bei *Säugetieren* (Kaninchen) gewonnen (Versteegh, 1927; de Kleyn u. Versteegh, 1933).

Es ist also für Vertreter aller Wirbeltierklassen erwiesen, dass die *pars superior* das Gleichgewichtsorgan ist; *Sacculus und Lagena* haben mit der statischen Funktion nichts zu tun. Da sie aber mit ihren ansehnlichen Nervenendstellen gewiss keine funktionslosen Teile sind, so ist es wahrscheinlich, dass sie dem Gehörsinn dienen—auch bei jenen Wirbeltieren, wo es bisher experimentell noch nicht erwiesen ist.

(2) Die *Beziehungen zwischen Bau und Funktion* sind für das Gehörorgan der Fische erst teilweise geklärt. Allgemein finden wir bei ihnen im Sacculus und in der Lagena ein Polster von Sinneszellen, dem seitlich der Otolith anliegt (vgl. Abb. 5–8, S. 222, 223). Bei eintretenden Vibrationen hat offenbar der *Otolith* für die mechanische Erregung der Sinneszellen hier eine ähnliche Bedeutung, wie die *Membrana tectoria* für die Erregung der Hörzellen in der Schnecke. Aber die Vibrationen können auf zweierlei Art zustande kommen, *ohne* und *mit* besonderen Hilfseinrichtungen. Dem entsprechen zwei Bautypen.

Den ersten Typus sehen wir in der Lagena der Ostariophysen und im Sacculus und in der Lagena der Nicht-Ostariophysen verwirklicht¹. Der scheibenförmige Otolith ist durch seine Randfaserung seitlich von der Nervenendstelle so schwebend befestigt, dass er seine Breitseite der dünnen Seitenwand der Schädelkapsel zuwendet. Von hier auftreffende Schallwellen werden zu einer Relativbewegung zwischen der trägen Masse des Otolithen und dem ihm anliegenden Sinnespolster führen. Dass schon eine primitive Anordnung solcher Art wirksam sein kann, scheint mir aus einer Beobachtung hervorzugehen, die ich selbst wiederholt gemacht habe: der Hupenton eines nicht zu weit entfernten Autos kann für den Tastsinn unserer Hand deutlich fühlbar werden, wenn diese eine unter dem Arm getragene Mappe berührt. Über ähnliche Erfahrungen an Taubstummen berichtet Kietzmann (1927): Wenn ein solcher einen Karton berührt, ist er zuweilen imstande festzustellen, dass in seiner Nähe gesprochen wird, während ihm ohne dieses Hilfsmittel selbst lautes Schreien in nächster Nähe nicht zum Bewusstsein kommt.

¹ Ich muss hier daran erinnern, dass für sie eine Horfunktion sehr wahrscheinlich, aber noch nicht exakt nachgewiesen ist (vgl. S. 227 und 232).

Ähnlich wie hier der feste Gegenstand für den Tastsinn der Haut, dürfte im Labyrinth der Otolith für die anliegenden Sinneszellen die Rolle eines Reizverstärkers spielen. Es ist anzunehmen, dass die Form der Otolithen und ihre Befestigungsweise dieser Aufgabe angepasst ist. Es wird kein Zufall sein, dass der Otolith des Utriculus mit seiner *statischen* Aufgabe durchwegs anders geformt und anders gelagert ist als der Otolith des Sacculus oder der Lagena mit seiner *akustischen* Aufgabe. Den Feinheiten seiner Konstruktion nachzugehen, wäre ein dankbares Problem für einen physikalisch geschulten Untersucher.

Den zweiten Typus finden wir im Sacculus der Ostariophysen, der durch die Weberschen Knöchelchen mit der Schwimmblase verbunden ist (vgl. S. 224). Dass eine Gasblase im Wasser unter dem Einfluss von Schallwellen, also unter dem Einfluss von aufeinanderfolgenden Verdichtungen und Verdünnungen des Mediums, in pulsierende Bewegung gerät, ist nicht nur theoretisch zu erwarten, sondern auch tatsächlich beobachtet (du Bois-Reymond, 1917). Dieser Vorgang muss auch bei der mit Gas gefüllten Schwimmblase der Fische vorausgesetzt werden. Er wird oft dadurch begünstigt, dass die Blase seitlich bis dicht unter die Haut heranreicht. Wir haben (S. 224) die Einrichtungen kennen gelernt, durch die bei den Ostariophysen diese Vibrationen von der Schwimmblase auf den Sacculus-Otolithen übertragen werden und die besondere Form dieses Otolithen besprochen (S. 225), durch die er zu einem Auffänger für die zugeleiteten Schallwellen gestaltet ist. Dieser Apparat scheint dadurch, dass er heftigere Vibrationen des Otolithen erzeugt, dazu geeignet, die Hörschärfe zu steigern (vgl. S. 233, 234). Nur er bedarf für die Zuleitung der Schallwellen zum Labyrinth einer perilymphatischen, von Bindegewebszügen freien Leitungsbahn, wie sie bei den Ostariophysen zum Sacculus ausgebildet ist und an der papilla basilaris aller anderen Wirbeltiere wiederkehrt. Bei einem Gehörorgan des ersten Typus, bei dem die Relativbewegung zwischen Otolith und Sinnespolster durch die Schallwellen direkt erzeugt wird, ist eine solche perilymphatische Leitungsbahn entbehrlich. Ich kann daher de Burlet nicht zustimmen, wenn er jedes Labyrinth ohne perilymphatische Leitungsbahn für einen rein statischen Apparat hält (vgl. de Burlet, 1928, 1929).

Eine perilymphatische Leitungsbahn kann auch bei einem Gehörorgan, welches nach dem Gasblasenprinzip arbeitet, überflüssig sein, und zwar dann, wenn die Gasblase der pars inferior unmittelbar anliegt. Ich komme hiermit auf einen eigenartigen anatomischen Befund, der für die Familie der Mormyriden¹ seit langer Zeit bekannt ist, aber in seiner physiologischen Bedeutung nur als völlig unverständlich hingestellt wird. Sie haben jederseits im Schädel, unmittelbar neben der lateralen Wand des Sacculus, ein gasgefülltes Bläschen mit fester, elastischer Wand. Diese kleinen Blasen entstehen entwicklungsgeschichtlich vom vorderen Pol der Schwimmblase (Abb. 17), lösen sich aber dann vollständig von ihr los und liegen isoliert am Labyrinth (vgl. de Beaufort, 1909; Bütschli, 1934, S. 648). Sie werden unter dem Einfluss von Schallwellen ebenso wie die Schwimmblase in pulsierende Bewegung geraten und diese auf den Sacculus übertragen. Durch die nachbarliche Lage ist hier eine Leitungsbahn, wie sie bei den Ostariophysen besteht, entbehrlich.

¹ Afrikanische Süsswasserfische von abenteuerlicher Gestalt.

Ähnliche Verhältnisse scheinen bei den Elopiden, Hyodontiden, Notopteriden und einigen anderen Familien zu bestehen. Hier bleiben aber die der pars inferior angelagerten Bläschen mit der Schwimmblase in offener Verbindung.

Ganz merkwürdige Verhältnisse trifft man bei den Clupeiden (Heringsfischen) an. Hier ziehen Verlängerungen der Schwimmblase nach vorn in den Schädel und bilden bläschenförmige Auftreibungen, die aber zum *Utriculus* in Beziehung treten (zuletzt untersucht von Wohlfahrt, 1935). Die topographischen Umstände und die abweichende Gestaltung des Utriculus-Otolithen lassen die Vermutung aufkommen, dass in diesem einzigartigen Falle auch der Utriculus teilweise als Gehörorgan dient. Der Einwand, dass die Verbindungskanäle zwischen der Schwimmblase und den am Labyrinth gelegenen Bläschen zu eng sind, um Schallwellen zu übertragen, ist nicht stichhaltig. Denn nach unserer Auffassung sind ja die kleinen Bläschen selbst die wirksamen Gebilde. Ob ihre Verbindung mit der Schwimmblase erhalten bleibt oder—wie bei den Mormyriden—obliteriert, dürfte keine unmittelbare funktionelle Bedeutung haben; massgebend hierfür dürfte sein, ob die kleinen Bläschen die Fähigkeit zu selbständiger Gassekretion und Gasregulation erworben haben; wo dies nicht der Fall ist, ist die offene Verbindung mit der Schwimmblase zur Erhaltung der Gasfüllung notwendig; es genügt aber für diesen Zweck natürlich ein ganz enger Kanal.

Es scheinen also bei einigen Nicht-Ostariophysen Einrichtungen vorzukommen, die in ähnlicher Weise wirken, wie die Schwimmblase der Ostariophysen, und wohl so wie diese die Hörschärfe steigern. Leider ist über das Hör-

vermögen dieser dem Experiment schwer zugänglichen Fische nichts bekannt¹.

(3) Mehrfach ist die Meinung geäußert worden (so von Sörensen, 1894–5; von Tait, 1932), die Schwimmblase der Ostariophysen sei ein *Resonanzapparat* und als solcher auf einen gewissen Ton abgestimmt, für den der Fisch besonders empfindlich wäre. Diese Annahme ist von vornherein unwahrscheinlich und experimentell nicht begründet. Versuche über die Hörschärfe in verschiedenen Tonlagen sind bisher nur an der Elritze ausgeführt worden (vgl. S. 220). Sie geben keinen Anhaltspunkt für eine auffallende Steigerung der Empfindlichkeit in einem beschränkten Tongebiet.

(4) Die *Helmholtzsche Theorie* sieht in der *Basilarmembran* der Schnecke einen peripheren Analysator, der durch die abgestufte Länge der Fasern auf verschiedenen hohe Töne abgestimmt ist. Da die Fasern der Basilarmembran mit verschiedenen,

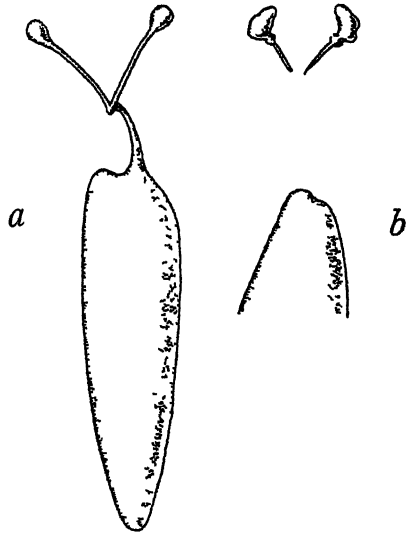


Abb. 17. (a) Entwicklung zweier Gasblasen vom vorderen Pol der Schwimmblase bei Mormyriden (*Gymnarchus*), bei einem Embryo von 34 mm. Länge. (b) Die losgelosten Enden der Fortsätze des erwachsenen Tieres, die sich an das Labyrinth anlegen (Nach Ballanthyne aus Butschli, etwas vereinfacht.)

¹ Siehe Anmerkung bei der Korrektur, S. 246.

getrennt abgeleiteten Sinneszellen in Verbindung stehen, bietet die Resonanztheorie eine Erklärung für die Fähigkeit der Tonunterscheidung. Dem Fischlabyrinth fehlt ein peripherer Analysator. Trotzdem kann auch der Fisch verschieden hohe Töne unterscheiden (S. 217). Aber dies ist kein Argument gegen die Helmholtzsche Hypothese. Wir müssen die Leistungen genauer betrachten.

Die Elritze kann durch das Ohr Töne, die um etwa eine Oktave auseinander liegen, noch sicher unterscheiden. Dieselbe Unterscheidung leistet ihr Hauttastsinn: Nach Ausschaltung des Labyrinths reagiert sie nur mehr im tiefen Tonbereich, hier aber lernt sie auch durch den Tastsinn der Haut Oktaven-Intervalle sicher unterscheiden (v. Frisch und Stetter, 1932). Um Frequenzunterschiede von dieser Grössenordnung wahrzunehmen, ist ein peripherer Analysator nicht notwendig. Der Tastsinn der menschlichen Haut leistet bei der Wahrnehmung von Vibrationen dasselbe, ja bei geschickter Versuchsanordnung und entsprechender Übung noch wesentlich mehr. Die Grundlage dieser Funktion ist sowohl beim Tastsinn der Haut wie beim Gehörsinn der Fische *die Fähigkeit dieser Sinnesorgane, Reize von hoher Frequenz ohne Verschmelzung dem Zentralnervensystem zuzuleiten*.

Die Möglichkeit der Tonunterscheidung auf diesem Wege hat aber ihre Schranken. Bei vielen Versuchen, die in unserem Institut ausgeführt worden sind, war die beste Leistung einer Elritze die sichere Unterscheidung zweier Töne mit einem Frequenzunterschied von 19 % (kleine Terz d^1/f^1). Durch den Tastsinn der menschlichen Haut können Frequenzunterschiede von 10–35 % noch erkannt werden (Katz, 1925; Gault, 1927; Knudsen, 1928). Durch das *menschliche Ohr* aber kann noch eine Differenz von 0.3–1 % wahrgenommen werden (Schaefer und Giesswein, 1926, S. 500; Gault, 1927; Knudsen, 1928). Das Ohr des Menschen ist also für Frequenzunterschiede viel empfindlicher als die menschliche Haut, die Fischhaut und das Fischohr, die untereinander in ihren analytischen Fähigkeiten auf etwa gleicher Stufe stehen. *Diese Steigerung der qualitativen Leistung ist wahrscheinlich auf die Erwerbung der Basilarmembran zurückzuführen*.

Die *quantitativen Leistungen* des Gehörorgans sind schon bei manchen Fischen auf einer erstaunlichen Höhe. Die Hörschärfe der Ostariophysen ist von derselben Grössenordnung wie beim Menschen. Für *diese* Leistungen bedeutete also das Auftreten der Basilarmembran keine Steigerung.

(5) Wir haben die Tonwahrnehmung durch das Labyrinth als "*Gehörsinn*", die Wahrnehmung von Schallwellen durch die Haut als "*Tastsinn*" bezeichnet. Aus Versuchen am Menschen weiss man, dass die akustischen Empfindungen mit den vibratorischen Hautempfindungen psychologisch nahe verwandt sind (vgl. z. B. Kietzmann, 1927; Petzoldt, 1928; v. Hornbostel, 1930). Katz (1925) sieht in den Vibrationsempfindungen der Haut eine Etappe der Entwicklung, die vom Tastsinn zum Gehörsinn des Menschen führt. Hier fügt sich der Gehörsinn der Fische als neues Glied in die Reihe der Übergangsstufen. Denn ihr Labyrinth ist gegenüber dem Tastsinn der Haut ein Apparat von gesteigerter und erweiterter Empfindlichkeit, der die Perzeptionsgrenze für Schallwellen hoch in die oberen Tonlagen verschiebt. Aber eine Verfeinerung der *Tonunterscheidung* bleibt den Gehörorganen mit Basilarmembran vorbehalten.

V. DIE BIOLOGISCHE BEDEUTUNG DES GEHÖRSINNES DER FISCHÉ

Lautäusserungen sind ein Mittel zu gegenseitiger Verständigung und werden in der Regel nur bei Tieren beobachtet, die einen *Gehörsinn* haben. Die sprichwörtliche "Stummheit der Fische" ist oft als ein biologischer Beweis gegen ihr Hörvermögen genannt worden. Aber nicht jedes Sprichwort ist zutreffend. Dass alle Fische stumm sind, ist bestimmt nicht richtig. Nach neueren Beobachtungen scheint vielmehr die Frage berechtigt, ob nicht die meisten Fische Töne erzeugen.

Viele von ihnen besitzen besondere Apparate zur Tonerzeugung. Dies wissen wir durch eine schöne Arbeit von Sörensen (1884), die leider dänisch geschrieben und darum wenig bekannt ist. Über ihren wesentlichen Inhalt hat kürzlich Jacobs (1935) in deutscher Sprache berichtet und gute Abbildungen gebracht. Knarrende und knackende Geräusche werden oft so hervorgebracht, dass besonders gestaltete Knochenflächen der Flossenstrahlen oder der Kiemendeckel an benachbarten Knochenflächen gerieben werden. Brummende, manchmal sehr laute und weithin hörbare Töne werden in mannigfacher Weise dadurch erzeugt, dass eigene Muskeln die Schwimmblase zum Vibrieren bringen. Weiss (1914) führt etwa 50 Fischarten an, für welche die Fähigkeit der Tonproduktion erwiesen oder sehr wahrscheinlich gemacht ist. In seiner Liste sind die Ostariophysen, deren scharfen Gehörsinn wir ja kennen gelernt haben, auffallend zahlreich vertreten. Aber die berühmtesten tönenden Fische (Trigliden, Sciaeniden) sind Nicht-Ostariophysen—vielleicht *weil* diese einen schlechteren Gehörsinn besitzen und die Töne daher lauter sein müssen, um von den Artgenossen gehört zu werden.

Es unterliegt wohl keinem Zweifel, dass ein genaueres Studium die Liste der tonerzeugenden Fische noch sehr vergrössern wird. So zählt neuerdings Burkenroad (1931) allein für Louisiana 25 verschiedene marine Arten auf. Erst in jüngster Zeit hat Hardenberg (1934) festgestellt, dass *Therapon theraps* (Fam. Pristipomatidae), ein Bewohner der Mündungsgebiete ostindischer Flüsse, ausserordentlich laute Töne hervorbringt. Aber bei der Scharfhörigkeit der Ostariophysen können bei diesen auch leise Töne von Bedeutung sein. Hier dürfen wir noch auf manche Überraschung gefasst sein. Hat doch Dykgraaf (1932) entdeckt, dass die Elritze (*Phoxinus laevis*) regelmässig bei Beunruhigung deutlich hörbare piepsende und knackende Töne hervorbringt. Wenn dies bei einem von Liebhabern und Forschern so viel beobachteten Fisch bisher nicht bemerkt worden ist, so müssen wir sagen, dass über die Verbreitung solcher Fähigkeiten ein abschliessendes Urteil heute nicht möglich ist.

So lassen sich auch über die biologische Bedeutung des Hörvermögens der Fische nur Vermutungen äussern. Wenn bei den Sciaeniden die Lautäusserungen hauptsächlich zur Zeit der Fortpflanzung auftreten, und wenn hier der tonerzeugende Apparat meist nur dem Männchen zukommt (Smith, 1905), so dient er wahrscheinlich dem Anlocken des anderen Geschlechts. Es ist klar, dass bei allen Arten, die zeitweise oder dauernd in Schwärmen leben, das Hervorbringen von Tönen und ihre Wahrnehmung ein wichtiges Mittel des Zusammenhaltens sein kann. Aber auch für Fische, denen die Fähigkeit der *Lauterzeugung* fehlt, kann die

Fähigkeit der *Lautwahrnehmung* wichtig sein. Manches Beutetier mag sich durch ein Geräusch verraten, mancher Artgenosse durch den schmatzenden Nachbarn zu einem gefundenen Brocken gelockt werden, mancher Hochseefisch durch den Lärm der Brandung vor der gefährlichen Nähe der Küste rechtzeitig gewarnt sein—aber hiermit kommen wir vorläufig aus dem Gebiet begründeter Theorien in das weite Reich der Phantasie.

VI. ZUSAMMENFASSUNG

1. Reaktionen auf Schallreize wurden bisher an 32 Fischarten (aus 14 Familien) zuverlässig nachgewiesen.
2. Spontane Reaktionen auf Töne sind nicht zu erwarten, da die von uns angewendeten Tonsignale für die Fische keine biologische Bedeutung haben. Zuverlässige Reaktionen erhält man daher nur nach *Dressur* auf Töne. Diese Methode ermöglicht auch eine weitgehende Analyse des Hörvermögens bei Fischen.
3. Die obere und untere *Hörgrenze* ist bei Fischen mit gut entwickeltem Gehörsinn angenähert dieselbe wie beim Menschen.
4. Die Fähigkeit der *Tonunterscheidung* ist für Elritzen (*Phoxinus laevis*) und Zwergwelse (*Amiurus nebulosus*) sicher nachgewiesen. Bei einem Intervall von etwa einer Oktave wurden zwei verschieden hohe Töne im Gedächtnis behalten und wiedererkannt. Der beste Fisch lernte sogar die Unterscheidung einer kleinen Terz. Auch mehr als zwei (bis zu fünf) Töne können gleichzeitig im Gedächtnis behalten werden.
5. Die *Hörschärfe* ist bei den geprüften Cypriniden, Siluriden und Characiniden angenähert dieselbe wie die des menschlichen Ohres.
6. Bei der Elritze (*Phoxinus laevis*) ist die pars inferior des Labyrinths, also Sacculus und Lagenae, der Sitz des Gehörsinnes. Die pars inferior hat keine statische Funktion. Die pars superior (Utriculus und Bogengänge) ist der Sitz des Gleichgewichtssinnes; dieser Teil des Labyrinths hat keine Hörfunktion.
7. Tiefe Töne (unter 100–150 v.d.) werden, wenn sie sehr intensiv sind, von der Elritze *auch* durch den Tastsinn der Haut, sehr tiefe Töne (16 v.d.) *nur* durch den Tastsinn wahrgenommen.
8. Bei den *Ostariophysen* (Cypriniden, Siluriden, Characiniden und Gymnotiden) steht die Schwimmblase durch die Weberschen Knöchelchen mit dem Sacculus in Verbindung. Der Sacculus-Otolith ist zum Auffangen der auf diesem Wege zugeleiteten Schallwellen besonders umgestaltet. Hierdurch erklärt sich die abweichende Form der pars inferior bei den Ostariophysen. Diese Einrichtung dient der *Steigerung der Hörschärfe*.
9. Die *Nicht-Ostariophysen* sind daher im allgemeinen für Schallreize weniger empfindlich. Dass auch sie durch die pars inferior des Labyrinths hören, ist noch nicht überzeugend nachgewiesen, aber ausserordentlich wahrscheinlich.
10. Auch bei manchen Nicht-Ostariophysen finden sich Einrichtungen, die der Steigerung der Hörschärfe dienen dürften. Sie sind aber physiologisch noch nicht untersucht.

11. Das Labyrinth der Fische vermittelt eine Tonwahrnehmung und Tonunterscheidung *ohne Basilarmembran*. Die Basilarmembran im Ohr der Landwirbeltiere ist wahrscheinlich ein Apparat zur Verfeinerung des Tonunterscheidungsvermögens.

12. Die Fähigkeit der *Tonerzeugung* dürfte bei Fischen sehr weit verbreitet sein. Daher ist auch die biologische Bedeutung ihres Hörvermögens nicht so rätselhaft, wie sie früher erschien.

VII. SUMMARY

1. Reactions to sound stimuli have so far been reliably demonstrated in 32 species of fishes (14 families).

2. Spontaneous reactions to musical tones are not to be expected, since the sound signals used by us have no biological significance for fishes. Reliable reactions can therefore only be obtained by conditioning to tones. This method also allows of a thorough analysis of the capacity of hearing in fishes.

3. The upper and lower limit of hearing in fishes that have a well-developed capacity of hearing is approximately the same as in man.

4. The capacity of discrimination of frequencies has been shown certainly to exist in minnows (*Phoxinus laevis*) and in a cat-fish (*Amiurus nebulosus*). Two different frequencies about an octave apart could be remembered and recognised. The best fish learned even to discriminate a minor third. And more than two (up to five) tones can be remembered at the same time.

5. In the Cyprinidae, Siluridae and Characinidae tested, the sensitiveness of hearing is approximately the same as that of the human ear.

6. In the minnow (*Phoxinus laevis*) the pars inferior, that is the sacculus and the lagena, is the seat of the sense of hearing. The pars inferior has no static function. The pars superior (utricle and semicircular canals) is the seat of the sense of equilibrium; this part of the labyrinth has no auditory function.

7. In the case of the minnow, low frequencies (below 100–150) are perceived also by the touch sensitivity of the skin if they are of high intensity, while very low frequencies (16) are perceived by the touch sense only.

8. In Ostariophysi (Cyprinidae, Siluridae, Characinidae and Gymnotidae) the swim bladder is linked up with the sacculus by the Weberian ossicles. The saccular otolith is specially modified for the reception of the sound waves directed towards it by the above mechanism. That explains the special shape of the pars inferior in the Ostariophysi. These dispositions are responsible for the increase in sensitiveness of hearing.

9. Fishes other than the Ostariophysi are therefore generally less sensitive to sound stimuli. It has not yet been convincingly proved that these fishes also hear by means of the pars inferior of the labyrinth, but this is very probably the case.

10. In some of the non-Ostariophysi there are, nevertheless, structures which may serve in increasing the sensitiveness of hearing. These have not yet been investigated physiologically.

11. The labyrinth of fishes has the capacity of the reception of sound and the discrimination of tones, though it has no membrana basilaris. The membrana basilaris in the ear of land vertebrates is probably an organ for the refinement of tone discrimination.

12. The capacity of sound production appears to be very common in fishes. The biological significance of their ability to hear is therefore not so puzzling as it previously appeared.

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Anmerkung bei der Korrektur (S. 239¹)

Inzwischen hat Herr Diesselhorst an unserem Institut Versuche an einem Mormyriden (*Marcusenius*) ausführen können. Die vermutete besondere Hörschärfe hat sich bestätigt.

ARGINASE

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I. INTRODUCTION

WHILE the ideally comparative review would embrace all the known forms of life, the limiting factor of space must necessarily preclude the attainment of this ideal, and the scope of this article has therefore been restricted to animals, fungi and bacteria. It is proposed to discuss the biological significance of arginase along rather broad lines in preference to giving an encyclopaedic account of the properties and possible functions of the enzyme. From a considerable mass of literature it has therefore been necessary to select for special consideration the papers which bear upon general rather than particular problems, and in this way it has been possible to trace some interesting correlations. Although a good deal of work has been devoted to the study of arginase as an intracellular enzyme, similar in many respects to other enzymes such as kathepsin and phosphatase, and although in view of our ignorance of intracellular processes in general this is a problem of great biochemical importance, it must be almost wholly neglected here. Again, the study of arginase in relation to the metabolism of tumours promises to lead to results of considerable importance, but this work too cannot be given more than incidental mention here. As key references to the literature of these aspects of the subject the papers of Waldschmidt-Leitz, Scharikova & Schäffner (1933),

¹ The author wishes to express his gratitude to the Royal Commission for the Exhibition of 1851 for a Senior Studentship during the tenure of which this article was written.

Klein & Ziese (1932 *a, b, c*, 1933) and the numerous publications of Edlbacher and his collaborators may be quoted.

Arginase is an enzyme which is very widely distributed among animal tissues. Many bacteria contain enzymes capable of decomposing arginine but not, as does mammalian arginase, with the production of ornithine and urea. I propose in what follows to reserve the name arginase for enzymes which catalyse the hydrolysis of *d*-arginine to give ornithine and urea. Arginase, in this proper sense, is one of the most specific enzymes known. The following substances, all of which are more or less closely related to arginine, have been shown not to be attacked by mammalian arginase:

Guanidinoacetic acid (Edlbacher, 1917; Edlbacher & Bonem, 1925; Dakin, 1907; Clementi, 1916).

β -guanidinopropionic acid (Edlbacher, 1917; Edlbacher & Bonem, 1925).

γ -guanidinobutyric acid (Thomas, Kapfhammer & Flaschenträger, 1923).¹

ϵ -guanidinocaproic acid (Thomas, 1913).

Guanidine (Clementi, 1916).

Guanidinoglycylglycine (Clementi, 1916).

Creatine (Dakin, 1907; Clementi, 1915*b*; 1916).

Creatinine (Dakin, 1907).

Agmatine (Edlbacher & Bonem, 1925).

Tetramethylenediguanidine (Baldwin, 1934).

α - δ -di-(*dl*-leucyl)-*dl*-ornithine (Abderhalden & Sickel, 1929).

Even *l*-arginine is not attacked (Riesser, 1906; Edlbacher & Bonem, 1925).

Both *d*- and *l*-arginine are split if perfused through a surviving liver (Felix & Morinaka, 1923), but this would probably involve deamination and the production of the optically inactive argininic (α -oxy- δ -guanidinovaleric) acid which, as Calvery & Block (1934) have shown, is almost as readily hydrolysed by arginase as is *d*-arginine.

This latter observation shows that the presence of the free α -amino group is not essential for the activation of the molecule, though it might have seemed otherwise from the statement of Steib (1926) that *dl*- α -*N*-methylarginine is resistant to the action of arginase. On the other hand, the carboxyl group of the molecule must be free, for neither the methyl ester of arginine (Edlbacher & Bonem, 1925; Calvery & Block, 1934) nor the ethyl ester of argininic acid (Calvery & Block, 1934) is hydrolysed, while only 50 per cent. of the available urea is split from arginylarginine (Edlbacher & Bonem, 1925; Edlbacher & Burchard, 1931), and this comes from the arginine residue of which the carboxyl group is free. Probably the guanidine residue also must be unmodified, since arginine phosphoric acid is not attacked (Meyerhof & Lohmann, 1928) neither are δ -*N*-methylarginine (Thomas, Kapfhammer & Flaschenträger, 1923) and δ -guanidino-*N*-caproic acid (Steib, 1926). The probability that both the carboxyl and the guanidine groups must be free is further emphasised by the fact that neither clupein nor clupeon is attacked by

¹ These authors retract the earlier statement of Thomas (1913) that γ -guanidinobutyric acid is hydrolysed by liver juice.

arginase (Kossel & Dakin, 1904*b*), while clupeon can pass through the liver unchanged (Felix & Morinaka, 1923). These results are confirmed by the observations of Lieben & Lieber (1934).

Mammalian arginase has frequently been used as a reagent for the detection and estimation of arginine as, for example, by Meyerhof (1928), Hunter & Dauphinee (1930 *a, b*), Baldwin & Needham (1933), Arnold & Luck (1933) and others. It has usually been assumed in such cases that the specificity of arginase is absolute, but the application of a method based upon such an assumption to a material about which we know practically nothing is liable to give misleading results; we do not know for certain that arginase is absolutely specific, nor do we know whether or not such material contains any potential substrate other than arginine. It is to be urged that when new substances related to arginine are isolated from invertebrate tissues, the action of arginase upon them should be immediately tested if there is any likelihood whatever that they may be attacked.

Work on the kinetics of the arginase-arginine system is difficult, since arginase is very labile under most conditions. It is fairly stable near the neutral point at ordinary temperatures, but with departure from neutrality is increasingly readily inactivated. Thus Hunter & Dauphinee (1933) found that at pH 5 or 10 about 50 per cent. of the activity of liver arginase is lost in only 10 min. at 37° C., and about 75 per cent. in an hour. At pH values less than 4 or greater than 12 inactivation is almost complete in 10 min. The optimum conditions of pH, temperature and time are interrelated variables, and in the case of arginase there is in practice an optimum neither of pH nor of temperature, but "at pH 9.8 the optimum for most conditions will be lower than 40° C. and for many lower than 30° C." according to Hunter (1934). Hunter & Morrell (1922) at first gave an optimum of pH 7.4, but later (1924) found the higher figure of 9.8. Edlbacher & Bonem (1925) found values of 9.5 and 9.8 at 26 and 38° C. respectively, while Hino (1926) obtained a figure of about 7.4. The more recent investigations of Hunter & Morrell (1933) make it certain that the optimum lies well on the alkaline side, near pH 9.8; the activity falls off sharply on the alkaline side on account of irreversible inactivation.

Gross (1920), working at pH 6.62 and 37° C., found that the velocity constants calculated from the equation for a monomolecular reaction fall off as the reaction proceeds, the reaction only going 70–85 per cent. towards completion. The composition of the reaction mixture was not altered in these experiments by the addition of fresh enzyme, and this appears to be because arginase is strongly inhibited by ornithine, though not at all by urea. According to Hino (1926) arginase is not affected by potassium bromide, iodide or cyanide or by quinine or atoxyl though, like most enzymes, it is destroyed by free iodine. Sodium fluoride inhibits and Waldschmidt-Leitz, Weil & Purr (1933) find that iodoacetate also inhibits, suggesting that the arginase system is in some way coupled to the oxidative systems of the cell. The results of Krebs & Henseleit (1932) indicate that the function of arginase in the intact cell is likewise dependent upon integrity of the cell structure and upon concomitant processes of oxidation.

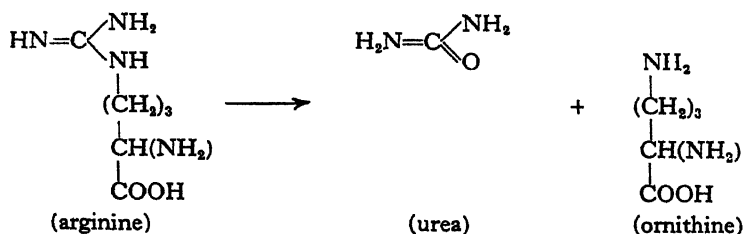
Work on the activation of arginase was begun by Salaskin & Solowjew (1931,

1932 *a, b*), and soon taken up by many workers. But the results which have been obtained contain some peculiar contradictions. Thus Klein & Ziese (1933) have shown that whereas crude preparations are inhibited by molecular oxygen this is not true of purified arginase. Similarly, while crude preparations are activated by cysteine, glutathione and hydrogen sulphide, these compounds produce only a marked inhibition of purified preparations. Meanwhile Waldschmidt-Leitz, Weil & Purr (1933) had come to the conclusion that the —SH group is an essential coferment and that “ohne dies ist Arginase wirkungslos”, a belief which was soon given up however (Purr & Weil, 1934). With Klein & Ziese (1933), Edlbacher, Kraus & Leuthardt (1933) point out that the behaviour of a given arginase preparation depends upon its previous history, and like Leuthardt & Koller (1934) point out that all the compounds which have an activating effect also possess reducing properties. They conclude that these activators, which include cysteine, glutathione, ascorbic acid, traces of iron, copper, manganese (Klein & Ziese, 1935), and even the unphysiological substance hydrazine (Klein & Ziese, 1933), act by a kind of *Schutzwirkung*, protecting the enzyme against the inhibitory action of oxygen. The problem, which is full of complications—see, for example, Karrer & Zehender (1934)—cannot yet be regarded as fully understood, and more work is necessary to resolve the many factors which would appear to be involved. From the more purely biological viewpoint it seems likely that normal tissues always contain sufficient activating substances to enable the enzyme to act at its full capacity within the cell, and that preparations in the form of a *Brei* probably give us a fairly reliable picture of the actual arginase content of any particular tissue.

II. THE OCCURRENCE OF ARGINASE

(1) *In the vertebrates*

The existence of a “urea-producing ferment” in the liver seems first to have been suspected by Richet (1894, 1897). Kossel & Dakin (1904*a*), however, were the first to show that urea is indeed present in such autolysates, and that it arises from arginine set free from the tissue proteins as a result of autolytic proteolysis. It was found that the enzyme, arginase, could be extracted with water and dilute acetic acid and precipitated from such watery extracts by means of ammonium sulphate, alcohol or ether, the precipitates showing a high degree of argininolytic activity. The enzyme, which was also present in press-juice, was found to catalyse the following reaction:



The authors pointed out that autolysates of liver do not contain arginine, and that this must be because the organ in question contains arginase, and, referring to the absence of arginine from autolysates of thymus (Kutscher & Seemann, 1901), intestinal mucosa (Kutscher & Seemann, 1902) and kidney (Dakin, 1903), wrote "das Fehlen des Arginins unter den Produkten der Autolyse lässt mit grosser Wahrscheinlichkeit auf die Gegenwart der Arginase in diesen Organen schliessen". Acting on this hint they proceeded (1904*b*) to examine various tissues of the calf, ox and dog. Liver, kidney, lymph glands and intestinal mucosa of all three animals gave positive results, muscle a doubtful positive, blood, spleen and adrenal negatives. By far the richest source of arginase was the liver. Shortly after this Mochizuki & Kotake (1904), who could find no arginine in autolysates of ox testis, suggested that this organ also might contain arginase and Mihara (1911) showed that such is indeed the case.

Little further progress was made until Clementi, armed with a new method (1914*a*), extended the search for arginase to a series of vertebrates other than mammals (1914*b*). The titrimetric method of Clementi, which was based upon formol titration, and which he afterwards modified (1922), was rapid and relatively sensitive; the major results which he obtained with it are summarised in Table I.

Table I

(Data from Clementi, 1914*b*)

Class	Species	Liver	Kidney
Mammalia	Dog	+	—
	Ox	+	—
	Pig	+	—
	Guinea-pig	+	—
	*Rat	+	—
	*Monkey (<i>Macacus rhesus</i>)	+	—
	*Man	+	—
Aves	<i>Gallus domesticus</i>	—	+
	<i>Columba livia</i>	—	+
	<i>Turtur turtur</i>	—	+
	<i>Fringuilla cloris</i>	—	+
Reptilia (Sauria)	<i>Lacerta agilis</i>	—	—
	<i>Anguis fragilis</i>	—	—
	<i>Coronella austriaca</i>	—	—
	<i>Emys europae</i>	+	—
(Ophidia) (Chelonia)		—	—
		—	—
Amphibia	<i>Rana esculenta</i>	+	—
	<i>R. temporaria</i>	+	—
Pisces (Elasmobranchii) (Teleostei)	<i>Torpedo ocellata</i>	+	—
	<i>Raia clavata</i>	+	—
	<i>Perca fluviatilis</i>	+	—
	<i>Abramis brama</i>	+	—
	<i>Barbus fluviatilis</i>	+	—

* Additional data from Clementi (1922).

The livers of mammals, a reptile, the frog and several fishes contained arginase, which could not however be detected in the liver of birds. It was found in the avian kidney and later (Clementi, 1915*a*) also in press-juices prepared from

mammalian kidney. Intestinal mucosa, spleen, testis, ovary and muscle from various animals contained no detectable amounts of the enzyme.

These findings led Clementi (1914*b*, 1915*a*) to enunciate the rule that arginase is present in the livers of animals having a ureotelic metabolism (mammals, chelonian reptiles, fishes, Amphibia) but absent where the metabolism is uricotelic in character (birds, sauropsid and ophidian reptiles). Felix & Tomita (1923) found that arginine is rapidly broken down to urea and ornithine when perfused through a surviving mammalian liver, but not in the case of the bird. In passing it is interesting to notice that Clementi's data (1915*a*) reveal the fact that of the fish livers studied, those of elasmobranchs were much more active than those of teleosts, while arginase was also found in the only elasmobranch kidney examined.

The technique of formol titration has also been used by Edlbacher (1915) in another comparative study of vertebrate tissues, the results of which agree fairly well with those of Clementi. Tissue suspensions were usually employed, but in some of the experiments press-juices were used with confirmatory results. The most important data are summarised in Table II. Mammalian thymus, spleen, testis,

Table II

(Data from Edlbacher, 1915)

Class	Species	Liver	Kidney
Mammalia	Calf	+	—
	Dog	+	—
	Rat	+	—
	Cat	+	—
	Guinea-pig	+	—
Aves	Fowl	—	—
	Pigeon	—	—
Reptilia (Sauria) (Ophidia)	Blindworm	—	—
	Viper	—	—
Amphibia	Frog	+	—

brain and intestinal mucosa were inactive, and very similar results were obtained by Fuchs (1921) who found arginase in human liver but not in the thyroid, pancreas, adrenal, spleen or kidney.

The next substantial step forward was made by Hunter & Dauphinee (1924*a, b*). Arginase was extracted from the tissues under examination by means of glycerol and the extract incubated with arginine under closely defined conditions of arginine concentration, *pH*, temperature and time. The relation between urea production and arginase concentration having been determined it was possible to express any given urea production in terms of an arbitrary unit of arginase concentration by reference to a standard curve. Urea was estimated by a colorimetric method elaborated by the same authors (1924*a*). The arginase activities, expressed as they were in terms of a purely arbitrary unit, had no absolute significance, but served nevertheless to give an excellent picture of the relative activities of different tissues. Table III presents the most interesting of the results; the numbers represent the

Table III

(Data from Hunter & Dauphinee, 1924*b*; see text)

Class	Species	Liver	Kidney
Mammalia	Cat	1280	2.7
	Rabbit	369	0.9
Aves	Hen	0	—
	Pigeon	0	18.3
Reptilia (Chelonia)	"Mud-turtle"	14.2	0.0
Pisces (Elasmobranchii) (Teleostei)	Dogfish (<i>Squalus sucklii</i>)	319	31
	Herring (<i>Clupea pallasii</i>)	181	7
	Other teleosts	8-110	1-5

number of arginase units per c.c. of the various glycerol extracts, all of which were prepared in a strictly uniform manner. Numerous organs other than the liver and kidney were examined in the course of this work and, with a few exceptions, uniformly negative results were obtained. But the heart and the muscles of the herring gave values of 8.8 and 0.9 units respectively, while the corresponding figures for the dogfish were 109 and 2.2 units. Furthermore, in the dogfish the only organs which were free from the enzyme were the brain, blood and ovum, although the latter contains a high concentration of arginase towards the end of development. The further researches of Hunter (1929) show that this wide dispersal of arginase in the tissues is characteristic of the elasmobranchs generally, since it was found both in the Batoidea and the Selachia. In contrast to these forms, the Holocephali, represented by the rat-fish, *Chimaera coliei*, appear to contain very little arginase, which is interesting since they are sometimes regarded as "a divergent and specialised offshoot from some primitive elasmobranch type" (Bridge, 1904). In *Chimaera* the liver and the heart contained at most only traces of arginase and the kidney only about 4 units per c.c. of extract.

Meanwhile the kinetics of the arginase-arginine system had received some attention, and Edlbacher & Bonem (1925) now worked at pH 9.5 in glycine buffer, decomposed the urea produced by means of urease, distilled off the ammonia set free and estimated it titrimetrically. Arginase was again found in mammalian (cat, mouse, dog, calf, guinea-pig, man) and in amphibian livers (frog), while bird livers (hen, pigeon) contained only traces, those of male birds containing more than those of females. Similarly the testis (cock, pigeon, ox, dog, guinea-pig) contained relatively more arginase than the ovaries (hen, pigeon, bitch); the testis of the calf resembled ovaries in arginase content. Mammalian kidneys contained traces of the enzyme (dog, rabbit, cat, mouse, guinea-pig) and the kidneys of birds also, but none could be demonstrated in the spleen (dog, cat, rabbit, mouse, guinea-pig, hen, pigeon), in the adrenal (guinea-pig) or intestinal mucosa (dog, cat, pigeon). Sendju (1925), whose comprehensive paper seems to be undeservedly little known, reported entirely similar results, except that the kidney, spleen and pancreas, as well as the liver, of a turtle proved to contain arginase.

Subsequent work has done little to modify these conclusions, though Ackermann (1932) found that the kidney, muscle, and especially the spleen of the ox show a slight argininolytic activity. Blood also may bring about a slight hydrolysis of arginine (Weil & Russell, 1934), but in all these cases it is a little difficult to feel certain that the observed results are really due to arginase. On the other hand, Hino (1924) found pancreatic juice to be free from arginase, while Edlbacher & Röthler (1925*b*) again found no evidence for its presence in the spleen, thyroid, pancreas, intestinal mucosa or muscle of any of the species which they examined, though traces were found in the thymus (calf) and placenta (rabbit, guinea-pig). That arginase is present in the placenta is suggested also by Salaskin, Solowjew & Tjukow (1932), who find that urea arises from arginine during placental autolysis: according to Solowjew & Mardaschew (1932) the arginase-arginine system is entirely responsible for the urea which appears during the autolysis of liver. On the whole we are probably justified in concluding that, in general, significant quantities of arginase are only to be found in the liver, kidney and testis.

The way was now prepared for further quantitative studies, and Edlbacher & Röthler (1925*a*) devised a method whereby it was possible to obtain numerical values having some degree of absolute significance for the relative arginase activities of different tissues. They adopted the use of urease followed by distillation and titrimetric estimation of ammonia for determining urea production. A standard glycerol extract of calf liver was prepared, and increasing quantities of this were incubated with arginine under standard conditions, the amounts of urea formed being determined in the manner just indicated. It was found that the activity of the enzyme, as judged by the amounts of urea formed, was not proportional to the enzyme concentration but fell off markedly as the latter increased. This could probably be accounted for if the enzyme were inhibited by the products of its action (cf. Northrop, 1920), and it is known that arginase is strongly inhibited by ornithine (Gross, 1920). Nevertheless, provided that the conditions were kept constant, the form of the activity-concentration curve was the same whatever tissue was employed, so that the original curve for calf liver could be used as a standard of reference. It was only necessary to define some convenient unit of arginase concentration in order to be able to interpret the urea production in any given case in terms of the corresponding arginase concentration, provided always that the standard conditions were rigidly observed. The unit chosen was that amount of enzyme which liberated an amount of urea corresponding to 0.34 mg. ammonia in 60 min. when incubated with 10 c.c. 1 per cent. arginine carbonate and 5 c.c. glycine buffer at pH 9.5 and 37° C., toluene being present as an antiseptic. By determining the amount of urea produced by a known amount of tissue under these conditions, the number of arginase units per gram of tissue (Edlbacher's *Arginase-wert*) could readily be calculated. It was found that the kidney arginase of fowls gave an almost linear activity-concentration relation, though that of the duck and the dove resembled that for calf liver, and Edlbacher & Röthler attribute this to the absence from fowl liver of some inhibitor which they suppose to be present in all the other tissues. It was necessary to construct a special standard reference curve

for this special case and in addition to define another arginase unit. This was accordingly done, but for all practical purposes the "kidney unit" (A.N.E.) and the original unit (A.E.) are identical. This method was now used (Edlbacher & Röthler, 1925*b*) in a study of the relationship between sexuality and arginase content.

The fowl and several mammals were examined. In the males the liver, kidney and testis were analysed and in the female only the liver and kidney. It was shown that other tissues do not contain significant quantities of the enzyme, so that the total arginase content of the whole animal could be approximately evaluated. Table IV summarises the results, which are expressed in terms of arginase units

Table IV

(Data from Edlbacher & Rothler, 1925*b*)

Animal	No. of exps.	Arginase units per gm. body wt.		♂ value as % of ♂
		Males	Females	
Dog	2	66	50	75
Cat	4	72	43	60
Rabbit	4	38	29	77
Guinea-pig	5	127	78	61
Rat	4	103	68	66
Fowl	20	0.363	0.277	63

Note that these data are not numerically comparable with those of Hunter & Dauphinee given in Table III.

per gram of whole body. In each case it appears that the total arginase content of female is only about two-thirds of that of male animals, and this relation holds as well for the birds as for the mammals in spite of the very great absolute difference between the two groups. The same is true if individual organs are considered; this is shown in Table VII, the data of which are calculated from Edlbacher & Röthler's data but are expressed in different terms, which will be explained later. Fujiwara's results (1929), obtained with mice and guinea-pigs, are entirely in support of those just discussed, and the influence of sexual factors is also shown in that chicks contain less arginase per unit weight than do adult fowls, while the arginase content of the testis of the ox increases at puberty (Edlbacher & Bonem, 1925).

(2) In the invertebrates

Hunter & Dauphinee (1924*b*) came to the conclusion that "arginase is an enzyme almost, if not entirely, peculiar to the vertebrates", a belief which closely fitted the current views on the distribution of arginine itself. The relevant facts have been exhaustively reviewed by Hunter (1928), Baldwin (1933) and by Kutscher & Ackermann (1933); for the present it is sufficient to say that until recently arginine was thought to be almost universally distributed in the invertebrates but replaced by creatine in the vertebrates. This, together with the few data summarised in Table V, gave the situation a misleadingly watertight appearance.

Table V. *Arginase in invertebrates*

Species	Part analysed	Arginase	Author
Termite larvae	Whole body	—	Clementi, 1918
Snail (<i>Helix pomatia</i>)	Hepatopancreas	+	"
Crayfish (<i>Astacus fluviatilis</i>)	"	—	"
Starfish (<i>Pisaster ochracea</i>)	Hepatic caecae	—	Hunter & Dauphinee, 1924b
Crab (<i>Cancer productus</i>)	Hepatopancreas	—	" "
Clam (<i>Saxidomus giganteus</i>)	Digestive gland	—	" "
Crayfish (<i>Astacus</i> ?)	Hepatopancreas	—	Ackermann, 1932 "

As Clementi (1930b and earlier papers) pointed out, the presence of large amounts of arginase among the vertebrates seems always to be associated with the production of urea, but Baldwin & Needham (1934) came to the conclusion that the snail, *Helix pomatia*, probably makes the urea which it excretes from exogenous arginine, thus resembling the hen (Clementi, 1932b). This raised the question as to how far the excretion of urea by invertebrates in general can be attributed to the action of a tissue arginase on ingested arginine, since most invertebrates excrete a certain amount of urea (Delaunay, 1927, 1931¹). It was therefore desirable to develop a very sensitive method for the detection and estimation of arginase and to carry out a survey of the invertebrate phyla, and this was begun by Baldwin (1935a). Conditions were worked out under which the urea production per mg. of tissue was constant, provided that the total amount did not exceed 4 mg. urea under the standard conditions; the initial slope of the activity-concentration curve then gave the required arginase content in terms of urea per mg. tissue. Controls were carried out for preformed urea in the tissues and the solutions, urea being estimated manometrically by the method of Krebs & Henseleit (1932)². This is at least 100 times as sensitive as the distillation method used by Edlbacher & Bonem (1925) and by Edlbacher & Röthler (1925 a, b). Baldwin's results were expressed in terms of the Q_H notation of Krebs & Henseleit (1932), i.e. as c.mm. urea-carbon dioxide per mg. dry tissue per hour, the temperature being 28 instead of 37° C. as used by Krebs. This fact is expressed by using the symbol Q_H^{28} . It seemed better to use this mode of expression than to add yet another to the list of arbitrary units already defined. The results of Edlbacher & Röthler (1925b) can be expressed in the same terms, and some of them have been so recalculated and are given in Table VII. It is evident from Table VI, which presents the results obtained by Baldwin, that arginase is more widely distributed than was formerly supposed, and it would clearly be desirable to extend the observations to many other animals. The amounts of arginase present in organisms such as the crab and the starfish are very small, suggesting that it was probably on account of lack of sensitivity that some, at any rate, of the negative results listed in Table V were obtained by the earlier workers. But the values for the terrestrial and fresh-water gastropods are very striking, for they are of the same order as those for mammalian livers. The possible significance of this will be discussed later.

¹ *Biological Reviews*.

² A similar technique has been used independently by Weil & Russell (1934) in their studies of blood arginase. Their paper appeared shortly before the work of Baldwin (1935a) was completed.

Table VI

(Data from Baldwin, 1935a)

Phylum and class	Species	No. of specimens	Q_{11}^{25}
Craniata:			
Mammalia	Guinea-pig ♀ (<i>Cavia</i>)	2	750
Echinodermata:			
Asteroidea	Starfish (<i>Asterias rubens</i>)	2	1.5
Arthropoda:			
Crustacea	Shore crab (<i>Carcinus maenas</i>)	3	15
Mollusca:			
Lamellibranchiata	Mussel (<i>Mytilus edulis</i>)	8	0.0
	Queen scallop (<i>Pecten opercularis</i>)	8	0.0
	River mussel (<i>Anodonta cygnaea</i>)	8	2.8
Gastropoda	Whelk (<i>Buccinum undatum</i>)	2	0.0
	Periwinkle (<i>Littorina littorea</i>)	16	0.0
	Limpet (<i>Patella vulgata</i>)	9	0.0
	River snail (<i>Viviparus fasciatus</i>)	6	719
	Ram's horn (<i>Planorbis corneus</i>)	12	22.5
	Pond snail (<i>Limnaea stagnalis</i>)	24	635
	Common snail (<i>Helix aspersa</i>)	7	1235
	Roman snail (<i>Helix pomatia</i>)	2	6500
	Black "slug" (<i>Arion ater</i>) (starved)	3	1070
		10	11.1

The organs analysed were the liver and the hepatic caecae in the guinea-pig and the starfish respectively and the hepatopancreas in all other cases. The nomenclature of the non-marine forms follows Ellis (1926).

Table VII. *Arginase contents of some tissues, calculated from the data of Edlbacher & Röthler (1925b)*

	Liver		Kidney		Testis
	♂	♀	♂	♀	♂
Mammalia: Dog	3300	2200	7	27	20
Cat	2650	1450	16	17	8
Rabbit	1800	1200	95	77	1
Guinea-pig	3900	2200	16	16	1
Rat	3200	1900	42	5	7
Aves: Fowl	4	3	36	23	13

(The figures correspond to Q_{11}^{25} values and are calculated assuming a dry weight equal to 20 per cent. of the wet weight for all cases.)

(3) In the fungi and bacteria

There can be no doubt that many micro-organisms are capable of breaking down arginine, but in general it seems unlikely that arginase, properly speaking, is concerned in the process. Shiga (1904) announced that arginase is present in yeast, but his technique was poor. Edlbacher (1917), using the formol titration method, was unable to confirm Shiga's result, but it is of course possible that the two observers used different yeasts. Kiesel (1922) obtained agmatine and urea by

allowing an "arginase" preparation of *Aspergillus niger* to act upon tetramethylene-diguanidine (which is not attacked by mammalian arginase), while Sendju (1925) found approximately the expected amounts of ornithine and urea when *A. oryzae* was allowed to act upon arginine.

The evidence for the presence of arginase in bacteria is not at all conclusive. Ackermann (1909) found optically inactive ornithine among the products of putrefactive decomposition of *d*-arginine carbonate, while Hino (1924) found that ammonia was produced when cultures of *B. pyocyaneus* and *B. fluorescens* were incubated with arginine, and attributed this to the simultaneous action of arginase and urease. Bacteria killed with acetone showed an argininolytic activity which appeared to extend to *l*- as well as to *d*-arginine, but no arginase could be detected in the culture medium, nor in *Streptococcus*, *Staphylococcus*, *B. coli communis*, *B. dysenteriae shiga*, *B. typhi*, *B. paratyphi* and *B. prodigiosus*. Sendju (1925) also obtained negative results with *B. coli*. Kossel & Curtius (1925) followed up the

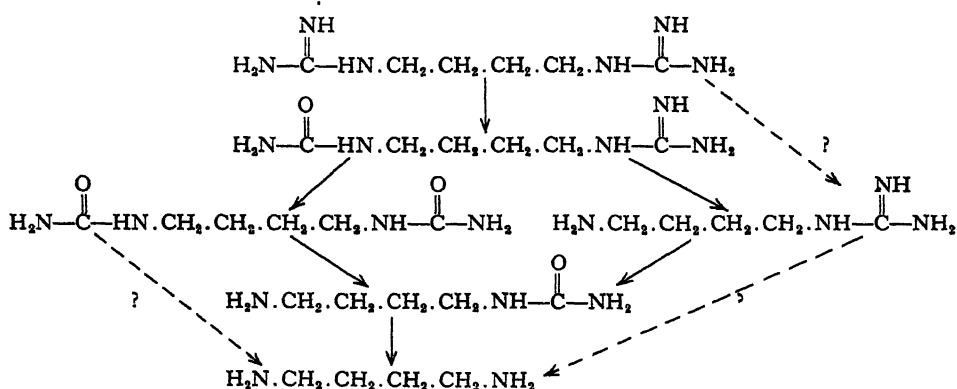


Fig. 1. Putrefactive decomposition of arcaine, after Linneweh—see text.

work of Hino, but only two of their results could possibly be regarded as definite evidence in favour of the presence of arginase, and these were both obtained with acetone preparations of one strain of *B. pyocyaneus*. Two other strains gave negative results, and the activity even of this one strain had disappeared after 3 months. Nevertheless, living bacteria of these strains were able to decompose *d*- and *l*-arginine, but as Kossel & Curtius themselves point out, the mere disappearance of arginine cannot be regarded as evidence for the presence of arginase. Hino (1924) had found that arginine may disappear under the action of certain bacteria and that, if it does, ammonia may be produced and the formol titration value of the solution be increased. But these facts could equally well be explained in another way.

The recent work of Linneweh (1931 *a*, *b*, 1932 *a*, *b*) is particularly suggestive in this respect. Linneweh studied the breakdown of arcaine under the influence of mixed cultures of putrefactive micro-organisms and found that it proceeds according to the scheme of Fig. 1. The final products are putrescine, ammonia, carbon dioxide and perhaps traces of urea (dotted lines in the diagram), while agmatine and a series of carbamyl compounds were isolated as intermediates.

Such changes as these were already known; thus agmatine was found by Reinwein & Kochinski (1924) to be convertible into putrescine under the influence of putrefactive micro-organisms, while creatine is converted into methylhydantoin (Ackermann, 1913 *a, b*; cf. Ellinger & Matsuoka, 1914) under similar conditions. Ackermann (1931) has also shown that arginine can be converted into citrullin in this manner and proposes the name "argininodesimidase" for the responsible enzyme, but Linneweh (1932*b*) suggests the more general term "guanidinodesimidase" for enzymes which convert the guanidine into the ureido group. Horn (1933), working with *B. pyocyaneus*, obtained 4.9 per cent. of citrullin from arginine, but could obtain none when *B. coli* was used, and these results compare interestingly with those of the earlier work which could probably be accounted for by guanidinodesimidase. We must suppose that many bacteria contain such an enzyme or group of enzymes. Even in the work of Linneweh the amounts of urea found were minute, and it seems, on the whole, that the existence of a bacterial arginase is an unnecessary postulate.

In conclusion it might be mentioned that Wada (1932) has prepared citrullin from casein by tryptic digestion but could find no reason for supposing that trypsin contains guanidinodesimidase, while Ackermann (1932) finds that the enzyme is probably entirely absent from mammalian tissues.

III. THE FUNCTIONAL IMPORTANCE OF ARGINASE

(1) *In ureotelic organisms*

It is to Clementi (1914*b*, 1915*a*) that we owe the first statement of the generalisation that arginase is associated with ureotelic metabolism. Ureotelic vertebrates excrete the ammonia which arises from the catabolism of proteins mainly in the form of urea. According to classical theory ammonium carbonate is first formed, undergoing two successive dehydrations to yield ammonium carbamate and then urea, but, while there has never been much doubt that urea is formed in the animal organism at the expense of ammonia and carbon dioxide, there has never been much evidence for the occurrence of ammonium carbamate as an intermediary. The chief alternative view has been that supported largely by Fearon (1926); here cyanic acid and ammonium cyanate were regarded as the intermediates, followed by tautomerisation of the cyanate to give urea as in Wöhler's classical synthesis. Here again the evidence is slight.

Ever since the discovery of arginase in 1904 it had been supposed that a part of the urea excreted by the mammals could be accounted for by the action of the hepatic arginase upon ingested arginine, but Krebs & Henseleit (1932) showed that the whole of the urea must derive from the action of this enzyme. The synthesis takes place in a cyclical manner, ornithine acting as a catalyst, in accordance with the scheme of Fig. 2. There is little need to go into any detailed discussion of the evidence for this scheme since the paper is already classical, but the reader may be referred to Krebs' review (1934) for further information.

The work of Manderscheid (1933) shows that the same cycle operates not only

in mammals but also in the livers of Amphibia (frog) and of chelonian reptiles (*Testudo graeca*) and even in the liver of the human foetus at 3-4 months, but not in avian liver. A logical basis has thus been established for the empirical rule enunciated by Clementi, which, as was shown in the first section of this article, applies without serious limitation to the vertebrate phylum, the ureotelic nature of the metabolism of chelonian reptiles (*Testudo* and *Emys*) having recently been confirmed by feeding experiments (Clementi, 1929*a*).

It is unfortunate that the fishes have not yet been at all thoroughly studied from this new viewpoint. Manderscheid (1933) has examined two fresh-water species (teleosts) and found no evidence for the presence therein of the ornithine cycle, but the results of Hunter & Dauphinee (1924*b*) and of Hunter (1929) show that the arginase content of teleostean fishes varies within wide limits. The elasmobranchs have not been examined at all from the new point of view, but they present a case of unusual interest and deserve brief mention here. Krukenberg (1888)

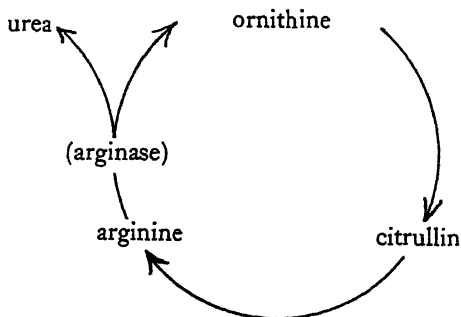


Fig. 2. Cyclical synthesis of urea in mammalian liver (simplified from Manderscheid, 1933).

showed that while urea is absent from members of the Cyclostomata, Cephalochordata and Teleostei it is abundantly present in the Elasmobranchii. This observation has repeatedly been confirmed; thus Kisch (1930) who, incidentally, gives an excellent summary of the earlier literature, found 1.36-2.54 per cent. of urea in the bloods of ten different elasmobranch species, while entirely similar values were found for the cerebrospinal, pericardial and perivisceral fluids, bile and ear lymph, as well as for the muscles and, in some species, the electrical organs. Urea is essential, moreover, for the maintenance of normal cardiac function in the elasmobranchs (Baglioni, 1906; Mines, 1912; Simpson & Ogden, 1932).

Like the marine teleosts, the elasmobranchs live in an environment the salinity of which is greater than their own, and they are therefore under the necessity of maintaining a constant internal salinity against an unfavourable osmotic gradient. The teleosts do this (see Smith, 1932) by continually swallowing sea water and actively excreting the unwanted salts, largely by means of special cells situated in the gill membranes. The elasmobranchs, however, meet the situation by raising the total osmotic pressure of the internal above that of the external environment by maintaining in it a concentration of about 2 per cent. of urea, to which the gills are practically impermeable. In the elasmobranchs, therefore, urea is not to be

considered simply as a metabolic end-product but as a highly soluble, non-toxic crystalloid upon which these fishes rely for the regulation of their internal environment. Correlated with this abundance of urea and the urgency of maintaining it we find that arginase is present in practically every organ of the body, often in high concentration. Kisch (1930), working on dehepatized elasmobranchs, came to the conclusion that the urea is by no means exclusively hepatic in origin, and this seems good reason for supposing that arginase, which is not here confined to the liver, is once more concerned with the production of urea, probably through the ornithine cycle. The results of Sendju (1925) suggest that a similar non-localised synthesis of urea may take place in chelonian reptiles.

Probably the Holocephali too would repay further study. We have already mentioned that Hunter (1929) found *Chimaera colliei* to be very poor in arginase, but Krukenberg (1888) states that he was able to isolate urea from the tissues of *C. monstrosa*. Further investigations would be interesting if only by reconciling these apparently contradictory findings, but it is likely that they would also provide new evidence respecting the much discussed ancestry of the Holocephali.

(2) *In uricotelic organisms*

We have noticed that the livers of birds and reptiles (excluding the Chelonians) are relatively poor in arginase. The reptiles have not been much studied, but it seems likely from the results of Manderscheid (1933) that the ornithine cycle is absent from the adult hen and also, according to Needham, Brachet & Brown (1935), from the embryo. The urea excretion of adult hens has been carefully studied by Clementi (1932 *a, b*), who finds that the output on a normal diet averages 70 mg. per day, falling to 20 mg. per day in starvation. The only substance which increases the output, apart from urea itself, is arginine, so that we must attribute the urea excreted by the bird to the action of arginase upon ingested arginine. Needham, Brachet & Brown (1935) have reached the same conclusion for the chick embryo. These results finally exclude the possibility suggested by Wiener (1902) that the formation of uric acid in the bird proceeds by way of urea. In the past a certain amount of evidence has been adduced in favour of Wiener's hypothesis, but in more recent times only negative results have been obtained as, for example, by Russo & Cuscuna (1931) and Cuscuna (1934) with the perfusion technique, and by Benzinger & Krebs (1933) using the tissue slice method. Nor is there any indication in the results of Schuler & Reindel (1933) that urea is in any way concerned in the production of uric acid. The subject has also been exhaustively studied by workers of the Italian school, but again with negative results. Thus urea has been given to birds (hens, geese, pigeons) by injection, the following substances being orally administered at the same time:

Malonic, tartronic and lactic acids (Clementi, 1929 *b*).

Pyruvic and propionic acids (Torrison & Torrison, 1931, 1932).

Dialuric and barbituric acids (Torrison, 1932, 1933).

Glycerol and glyceric acid (Biondi, 1931).

Tartronic, lactic, barbituric and dialuric acids (Russo, 1933).

In no case was there any significant increase in the amounts of uric acid excreted, and in some cases the urea was quantitatively recovered. Minkowski (1886) was the first to demonstrate that ammonium carbonate is converted into uric acid in the avian organism and this remains the only substance known to yield uric acid directly, whether in the adult bird (Clementi, 1930*a*, 1932*a*) or in the embryo (Needham, Brachet & Brown, 1935). Whatever the mechanism of uric acid synthesis in the bird may be it is at least certain that urea does not enter into the scheme and that arginase is not concerned in the synthesis.

But it has long been known that birds excrete benzoic acid, which is for them a dietary constituent of frequent occurrence, in the form of ornithuric acid, *i.e.* dibenzoylornithine (see Sherwin, 1922 and Lusk, 1928, in this connection), and Clementi (1914*b*, 1915*a*) pointed out that the ability to effect this synthesis must probably be associated with the presence of arginase in the avian kidney. Crowdle & Sherwin (1923) find that although hens can synthesise ornithuric acid on a protein-free diet, the only aminoacid capable of increasing the output of ornithine is arginine. Evidently, then, although arginase is probably of no importance in the main processes of nitrogenous metabolism in the bird, it is important for the production of ornithine for protective synthesis. It probably accounts also for all the urea excreted on a normal diet.

Of other uricotelic organisms we know little. The literature contains no information regarding the Insecta, but the gastropod molluscs have recently been studied a good deal from this point of view. The snail, *Helix pomatia*, was shown by Clementi (1918) to contain arginase, and it seemed to Baldwin & Needham (1934) more than coincidence that this animal was also practically the only invertebrate known to excrete significant quantities of urea (Delaunay, 1927, 1931). They confirmed Clementi's finding of arginase in the hepatopancreas and found it in the nephridium also, but were unable to demonstrate any synthesis of urea by surviving slices of the "liver". They were led to conclude that *Helix* resembles the bird, though the possibility of a synthesis could not be finally excluded. At this time, however, there existed no reliable numerical data as to the concentration of arginase in the hepatopancreas, and the subsequent investigations of Baldwin (1935*a*) reopened the question of a possible synthesis of urea, for it then appeared that the hepatopancreas of *Helix* contains as much arginase as the most active of the mammalian livers. The failure of Baldwin & Needham (1934) to demonstrate such a synthesis would be explicable if the urea were being transformed into some other substance as rapidly as formed.

Now there exist among gastropods remarkable correlations between habitat and the production of uric acid (Needham, 1935) and again between uric acid production and arginase content (Baldwin, 1935*a*). This suggests that arginase may be in some way concerned in the synthesis of the uric acid which is so characteristic a feature of the nitrogenous excretion of the terrestrial gastropods. It seems possible that urea is first formed, possibly through the ornithine cycle, and then converted into uric acid, perhaps by the mechanism suggested by Wiener (1902), *i.e.* by condensation with tartronic acid. We have seen that Wiener's scheme is

quite inadmissible for the case of the birds, but some experiments by Baldwin & Needham (1934) have shown that the production of uric acid probably follows entirely different paths in the snail and in the bird; furthermore, Baldwin (1935*b*) finds that the snail is able to convert urea and tartronic acid together into uric acid. It has been shown by Howes & Wells (1934) that the snail undergoes alternate periods of high and low water content, and that activity is always associated with a high degree of hydration. Baldwin suggests that in *Helix*, at any rate, there may also be an alternation of ureotelic and uricotelic types of excretion; when the water content is high urea may be excreted, but during periods of desiccation this urea, which cannot now be excreted, may be converted into uric acid and stored in that form to be evacuated later.

These speculations have received a certain amount of experimental support on the lines just indicated and, so far at least, do not appear to be contradicted by any of the known evidence. They would suggest that unlike the bird, which has developed entirely new mechanisms for producing the uric acid required by a cleidoic development and a terrestrial existence, the snail has modified a previously existing mechanism, urea being formed as the primary end-product of nitrogenous catabolism but being secondarily converted into uric acid. Thus although xerophilous vertebrates and gastropods alike are mainly uricotelic, convergent evolution, in the chemical sense, would appear not to imply the development of identical or even similar chemical mechanisms.

(3) *In growth*

Edlbacher (1917) detected arginase in foetal human liver as early as the fourth month, and Fuchs (1921) too has found it in the human foetus. According to Manderscheid (1933) the ornithine cycle is already present in the human foetus at the third or fourth month. Hunter & Dauphinee (1924*b*) found that, although absent from the unfertilised elasmobranch egg, the enzyme is abundantly present shortly before hatching when the embryo, according to Needham & Needham (1930), shows a very marked uraemia. Similarly Takahashi (1928) found that the conjugation of benzoic acid with ornithine can be accomplished as early as the ninth day by the chick embryo. Arginase, then, can discharge its adult function quite early in embryonic life. But it is present even earlier than this. Thus in the chick "the capacity to produce ornithine from arginine appears to be present considerably earlier than the capacity to conjugate it to form ornithuric acid" (Needham, Brachet & Brown, 1935). Edlbacher & Mertz (1927) again have detected arginase even in undifferentiated guinea-pig embryos. The latter authors also studied a variety of neoplastic growths and wrote: "wir gelangen zu dem Schluss, dass wir in dem Argininabbau... einen spezifischen Wachstumsfaktor zu erblicken haben, durch den sich auch das Gewebe maligner Säugertumoren von normalem Gewebe chemisch differenzieren lässt." If this is true we should expect to find that the arginase content of embryonic tissues progressively falls off as time goes on and the growth rate diminishes and Needham & Brachet (1935) have shown that this is actually the case in the chick. They give the following

average values for the arginase content of the embryo, the figure at hatching being calculated from the data of Chaudhuri (1927):

Day	Q^{ss}_H
2½	1.16
3	0.87
3½	0.63
8	0.09
12	0.04
Hatched chick	0.05

It is known, however, that the glutathione content (Murray, 1926) and the total reducing power (—SH *plus* ascorbic acid *plus* unknown reducing substances; Bierich & Rosenbohm, 1935) of the chick fall off as development proceeds, and it is also known that glutathione, cysteine and ascorbic acid will activate arginase under certain conditions. The observed diminution in arginase activity might therefore be due to the decrease in the amount of activators available, but no increase in activity was observable when glutathione, cysteine and ascorbic acid were added to embryos at 4½ and 8 days and to the fifth-day yolk-sac. These results lend valuable support to the theory of Edlbacher which, hitherto, had rested almost entirely upon work with tumour tissue; but they are also interesting when compared with certain results of Waldschmidt-Leitz & McDonald (1933). According to these authors, although necrotic tissue contains more arginase than the actively growing parts, the arginase activity of a tumour does not increase much as the tumour ages and the proportion of necrotic tissue increases, and this, as the results of Edlbacher, Koller & Becker (1934) show, is correlated with the presence of only very small amounts of reducing substances in the necrotic parts.

Fuchs (1921) used the technique of formol titration to study various pathological conditions including carcinoma of the liver, and found that the metastases contain arginase. Fujiwara (1929) has studied the effect on the arginase content of the host of implanting transplantable tumours in mice. Within 24 hours of implantation the average arginase content of the kidneys of a series of female mice had fallen from 312 to 65 units (Edlbacher's A.E.) and that of the livers from 4000 to 1400. While it is striking that there is such a profound effect upon the tissues of the host, the injection of arginine alone is said (Edlbacher & Schuler, 1932) to lead to a fourfold increase in the arginase content of the muscles and kidneys of normal guinea-pigs. It seems that these changes must be connected with general rather than specific metabolic processes.

Edlbacher & Mertz (1927) paid special attention to the activity of tumour tissues itself, and found that neoplastic tissues in general are characterised by a high argininolytic activity. It was upon these observations in the first place that Edlbacher based the generalisation that growing tissues as a whole possess a high arginase activity. In a series of later papers (Edlbacher & Kutscher, 1931, 1932; Edlbacher, Koller & Becker, 1934; Edlbacher & Jung, 1934; Edlbacher, 1934) Edlbacher and his collaborators have elaborated the view that arginase, nuclease and phosphatase are peculiarly associated with a high rate of cell division and

growth. It is held that arginase is not here concerned with the formation of urea, and this is upheld by the findings of Neber (cited in Edlbacher, Koller & Becker, 1934), but with the synthesis of the protein components of nucleoplasmic material, probably in connection with the large amounts of arginine which characterise the protamines and histones. That such a correlation between growth and high arginase content exists cannot be doubted, and it is probably significant in this connection that the testis, in which organ alone active cell division persists into adult life, contains more arginase than any other tissue, apart from the liver and the kidney, and, furthermore, that its arginase content increases suddenly at puberty (Edlbacher & Bonem, 1925).

IV. SUMMARY

1. Arginase is a highly specific intracellular enzyme and requires that both the carboxyl and the guanidine groups shall be free if the substrate molecule is to be activated. The kinetics of arginase and its behaviour towards activators and inhibitors are very briefly discussed in the text.

2. Arginase is abundantly present in the liver of ureotelic vertebrates; small amounts are present also in the kidney and testis, but little or none elsewhere. In uricotelic vertebrates it is mainly confined to the kidney.

3. Male animals always contain about 50 per cent. more arginase than females; a sudden increase in the arginase content of the testis at puberty suggests a possible specific relation between sex and the distribution of arginase.

4. Arginase is widely distributed among invertebrates, usually in small amounts. But the terrestrial gastropods contain as much of the enzyme as do the ureotelic vertebrates.

5. The synthesis of urea by ureotelic vertebrates takes place by a cyclical mechanism involving arginase. This system is present in mammals, chelonian reptiles and Amphibia, but not in birds. It is very probably present in the elasmobranch fishes also, and the possibility that it is present in the teleosts remains open.

6. Although arginase is not concerned in the production of uric acid by uricotelic vertebrates it does account for such urea as is excreted by these forms. Its main function in the birds is that of supplying ornithine for detoxication.

7. Many invertebrates resemble the birds in possessing small amounts of arginase, which probably suffice to account for the urea which they excrete. In the terrestrial gastropods it is possible that metabolism is primarily ureotelic as in the mammals, but that urea is secondarily converted into uric acid as an adaptation to xerophilous life.

8. Although many bacteria are capable of breaking down arginine they probably contain no arginase but only guanidinodesimidase. This enzyme appears to be absent from vertebrate tissues.

9. Arginase is present early in embryonic life and is soon capable of discharging its adult function. But there exists a correlation between a high growth rate and a high content of arginase, phosphatase and nuclease, which suggests a specific association between arginase and the processes of growth.

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DISEASE RELATIONSHIPS IN GRAFTED PLANTS AND CHIMAERAS

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I. INTRODUCTION

GRAFTING has been employed by a number of workers as an experimental method in investigating the nature of resistance and susceptibility to disease in plants. In some cases, the results of these experiments have been further applied in interpreting the so-called "graft hybrids" and chimaeras. The widespread use of grafting and budding in horticultural practice has aroused considerable interest in "stock-scion" effects, and, in recent years, the behaviour of a large number of different combinations has been recorded. With regard to altered disease relationships, however, most workers have been content with observations of a purely empirical nature. In this paper, an attempt is made to co-ordinate these observations, and to explain them, as far as possible, in terms of the factors underlying resistance and susceptibility in each case. In this way, an estimate may be formed of the value and limitations of the method of grafting in the investigation of plant-disease problems and the control of disease under field conditions.

Grafted plants and chimaeras are the subject of an extensive literature. Among the books and papers consulted in connection with the present survey, the most valuable in supplying further sources of information have been those of Winkler (1912) and Krenke (1933) for the general and experimental literature, of Hatton (1930) for a résumé of recent knowledge of "stock-scion effects" in relation to horticulture, and of Weiss (1930) and Neilson Jones (1934) on the subject of "graft hybrids" and chimaeras. In each of the above cases, a few paragraphs are devoted to the subject of disease relationships. Many of the references quoted in the present paper are scattered throughout horticultural and pathological literature and have not hitherto been collated.

II. THEORETICAL ASPECT

The majority of the early experimental investigations with grafted plants were concerned with the possibility of a direct reciprocal influence of stock and scion, for instance, by the translocation through the graft union of the substance or substances actually responsible for resistance or susceptibility to disease. These studies were necessarily confined to plants that are readily propagated by grafting, and diseases showing a high degree of specialisation were generally chosen. Thus, Fischer (1912) and Sahli (1916) investigated the effect of grafting on the susceptibility of various Pomaceae to infection by *Gymnosporangium* spp., as follows:

- (1) *Gymnosporangium confusum*: *Mespilus germanica* (immune) on *Crataegus oxyacantha* (susceptible).
- (2) *G. tremelloides*: *Sorbus aria* (immune) on *S. aucuparia* (susceptible).
- (3) *G. sabinae*: *Bollvilleria malifolia* (susceptible) on *Pyrus communis* (immune); *Bollvilleria auricularis* (susceptible) on *Sorbus aria* (immune).

In every case, both stock and scion retained their characteristic reaction unaltered. Similarly Roach (1923) found that when two different varieties of potato were grafted together at an early stage of their development, the reaction of the subsequently formed tubers to artificial inoculation with the wart disease organism, *Synchytrium endobioticum* (Schilb.) Perc., was entirely unaffected by the varietal reaction of the foliage. Later (1927) his results were extended to show that the tubers were also unaffected by the nature of the root system. Roach's original observations were confirmed by Köhler (1925) for plants infected naturally through the soil. The absence of any reciprocal influence of stock and scion, affecting disease relationships, was also reported by Gibson (1904) for chrysanthemum rust, caused by *Puccinia chrysanthemi* Roze, by Salmon and Ware (1927) for the powdery mildew of hops, caused by *Sphaerotheca humuli* (DC.) Burr, by May (1930) for the wilt of tomatoes, caused by *Fusarium lycopersici* Sacc., and by Leach (1929) for bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav.

Negative results would appear to indicate "...that, if resistance or susceptibility is due to a specific substance in the plant, this substance is not transmitted from stock to scion or *vice versa*, or else it is modified by the cells which receive it" (Leach, 1929). Roach, in his later paper (1927), refers to several well-known instances of the transmission of organic substances regarded as characteristic of stock or scion. For example, the alkaloids atropine and nicotine have been observed to pass from *Atropa belladonna* to *Solanum tuberosum*, and from *Nicotiana tabacum* to *N. affinis*, respectively. On the other hand, inulin, a soluble polysaccharide characteristic of *Helianthus tuberosus*, can pass from this species to *H. annuus* only in the altered form of dextrin. Resistance to wart disease in the potato is assumed to be a fundamental property of the protoplasm, and a further investigation of the complex proteins of immune and susceptible varieties is suggested.

An instance in which susceptibility to disease was apparently influenced by grafting was recorded by Blaringhem (1924). This author noted that the variegated

form of *Lavatera arborea* was almost entirely immune to infection by *Puccinia malvacearum* Mont., whereas the normal form was highly susceptible. Attempts to graft the latter on the former were unsuccessful, but six out of ten of the reciprocal grafts succeeded. In each case, shoots from the variegated scions were found to be equally susceptible as the stocks on which they were grafted, a fact which led to the conclusion that resistance was physiological rather than genetic in nature. There is no evidence, however, to show that a similar increase in susceptibility could not have been induced by other means than grafting on a susceptible stock. An hereditary effect of grafting in relation to root-nodule infection was described by Richmond (1926). The nodule organisms infecting the Lima bean (*Phaseolus lunatus*) and the navy bean (*P. vulgaris*) were specific for their respective hosts and unable to induce nodule formation on cross-inoculation. Although the specific adaptation was retained when the two species were grafted together, the seeds produced from the grafted plants were found, on germination, to be equally capable of infection by either organism. This was assumed to be a modification induced by grafting, but since the experiments were described in very inadequate detail, a more likely explanation would seem to be that the cultures were contaminated or that the plants themselves were segregating for the factor or factors concerned.

Several instances of the use of inoculation experiments in the interpretation of the so-called "graft hybrids" or plant chimaeras are quoted in the recent book by Neilson Jones (1934), but an extension of his observations appears desirable. In general, the supporters of the periclinal chimaera theory for such forms as *Crataegomespilus*, *Pirocydonia*, and the various *Solanum* graft hybrids have explained the disease relations of these plants on two suppositions, as follows:

(a) The absence of a reciprocal influence affecting the normal disease relationships of the components.

(b) The penetration, by fungi, of the epidermis and possibly of one or more subepidermal layers of plants otherwise immune to infection.

Thus, Fischer (1912) reported that *Crataegomespilus Asnieresii*, a reputed "graft hybrid" between *Crataegus oxyacantha* and *Mespilus germanica*, was susceptible to *Gymnosporangium confusum* Plowr., although, as mentioned above, the *Mespilus* itself was almost entirely immune and remained so when grafted on to *Crataegus*. Sahli (1916) confirmed this result, and showed that *Crataegomespilus Dardari* was susceptible also. However, this plant was not so readily infected as *Cm. Asnieresii*, nor was either of the *Crataegomespili* so completely susceptible as *Crataegus* itself. Here, the conclusion arrived at was that *Crataegomespilus Asnieresii* and *Cm. Dardari* were respectively monochlamydus and dichlamydus chimaeras, the outer layer in each case consisting of unaltered cells derived from the immune *Mespilus germanica*. Slightly different results were obtained with *Gymnosporangium clavariaeforme* Jacq., to which only *Crataegomespilus Asnieresii* was susceptible. Here, also, *Crataegus* was susceptible, but *Mespilus* was completely immune. In this case, adherence to the periclinal chimaera theory would require that the outer layers of *Mespilus* tissue presented an impassable barrier to the fungal germ tube. Actually, as Weiss (1930) and Neilson Jones (1934) point

out, the leaf of a dichlamydius chimaera may be composed almost entirely of the tissues of the outer component, in which case the susceptibility of this plant to *Gymnosporangium confusum* must depend on the presence of a small proportion of *Crataegus* tissue, coupled with the fact that the *Mespilus* itself is apparently not completely immune.

The conclusions of Fischer and Sahli were later contested by Maurizio (1927), on the results of inoculation experiments with a form of *Podosphaera oxyacanthae* (DC.) de By. Although both *Crataegus* and *Mespilus* were readily infected, *Crataegomespilus Dardari* was only slightly susceptible, and *Cm. Asnieresii* was entirely immune. Maurizio interpreted these results as being in favour of the "burdo" theory, both plants being true graft hybrids resulting from the actual union of vegetative nuclei. Their behaviour towards the *Gymnosporangium* spp. could also be explained on this basis rather than on the two assumptions mentioned above, but it was admittedly strange that their reaction to the two fungi should differ so widely. The same author also carried out infection experiments with *Pirocydonia* spp., believed to be graft hybrids between *Pyrus communis* and *Cydonia vulgaris*. Two forms were available, namely *Pirocydonia Danieli* and *Pc. Winkleri*, and both were slightly susceptible to *Gymnosporangium sabinae* (= *Aecidium cancellatum*), being in this respect intermediate between the pear, which was still more susceptible, and the quince, which was immune. *Pirocydonia Winkleri*, however, was only very slightly infected, and had previously been reported by Daniel to be immune (Weiss, 1930). On the other hand, experiments with a further form of *Podosphaera oxyacanthae*, to which both pear and quince were moderately susceptible, showed that *Pirocydonia Winkleri* was far more readily infected than either, whereas the infection of *Pc. Danieli* was once more intermediate in character. Maurizio was unable to adopt any definite conclusions from these results. Weiss, on the other hand, considers that "... there is every reason to regard *Pirocydonia (Danieli)* as a true graft hybrid" (1930, p. 250), while in the opinion of Neilson Jones (1934), it is a monochlamydius chimaera, the immune *Cydonia* epidermis being sufficient to prevent the formation of aecidia by *Gymnosporangium sabinae*. *Pirocydonia Winkleri*, moreover, is believed to be a "sport" derived entirely from the quince root-stock.

Inoculation experiments were also performed by Klebahn (1918) on various forms derived from a combination of *Solanum lycopersicum* with *S. nigrum*. These he believed to be of the following constitution:

S. tubingense: monochlamydius; epidermis of *S. lycopersicum*.

S. proteus: dichlamydius; outer layers of *S. lycopersicum*.

S. Kolreuterianum: monochlamydius; epidermis of *S. nigrum*.

S. Gaertnerianum: dichlamydius; outer layers of *S. nigrum*.

S. Darwinianum: inner core and epidermis of *S. nigrum*, separated by a layer of "burdo" tissue derived from the latter and *S. lycopersicum*.

Results with *Septoria lycopersici* Speg., to which the tomato only was susceptible, were explained by means of the two suppositions stated above. Moreover, two layers of susceptible tissue were considered sufficient for the abundant development of infection, the mycelium being able to penetrate into the resistant tissue under-

neath. Actually, this appearance may be due to the fact already mentioned, that the leaf of a dichlamydius chimaera may be derived almost entirely from the tissues of the outer component. No results were obtained with *Cladosporium fulvum* Cke, owing to conjectured loss of virulence in culture.

A further investigation of disease relations in problematical graft hybrids and chimaeras might lead to some interesting results. The possibility of their economic application has been recognised by Jørgensen (1928) in his attempts, so far unsuccessful, to raise a periclinal potato: tomato chimaera resistant to blight (*Phytophthora infestans* (Mont.) de By.). The chief difficulty in the theoretical interpretation of these experiments lies in the fact that the possibility of a reciprocal influence affecting the disease relations of the two components of a chimaera automatically introduces the possibility that the latter may no longer be distinguishable, as such, from the similarly modified "burdo" tissue, resulting from the fusion of vegetative nuclei, of a true graft hybrid. The same difficulty occurs in the interpretation of morphological or other data. The fact that the characteristic resistance or susceptibility of the two components may, in some cases, be unaffected by their juxtaposition is strongly, but not conclusively, suggested by results such as those of Klebahn (1918), already quoted, where the chimaeral structure of the plants in question had previously been deduced from other considerations. In this connection, experiments with grafted plants are particularly important, for if resistance or susceptibility can be shown to remain unaffected by grafting, there will be better justification for assuming that they are retained unaltered in a chimaera.

As early as 1912, Winkler (1912) suggested that grafting might affect disease relationships indirectly, as for instance by its effect on the extent of the root system, or on rate of transpiration, or other characters usually regarded as resulting from a response to environmental conditions. However, this possibility was apparently not considered in any of the experimental work until 1931, when Volk (1931) investigated the effect of grafting on the susceptibility of potato and tomato to potato blight (*Phytophthora infestans*) and tomato leaf mould (*Cladosporium fulvum*) respectively. Volk came to the conclusion that resistance and susceptibility were specific properties of stock and scion respectively and could hardly be transmitted as such, but might, on the other hand, be subject to modification as a result of nutritional and other disturbances. In his experiments, special emphasis was placed on the state of vigour of the host plant, which, under certain conditions, might have an adverse effect on the development of the parasite. On the other hand, immunity to disease was in no case affected. Although Volk's account of his experiments is by no means satisfactory, his work represents a point of view which had become increasingly prominent in horticultural literature, although almost entirely overlooked in experimental work.

III. PRACTICAL ASPECT

Grafting and budding have been used in horticulture since very early times for the propagation of deciduous and evergreen fruits and other useful and ornamental trees and shrubs. They have also been used in the direct prevention and control

of disease, as for instance by in-arching, bridge-grafting and top-working with suitable varieties to replace portions of the tree lost or damaged by disease or mechanical injury. Examples of the use of resistant stocks in the prevention of soil infection are to be found in several recent recommendations. Thus, Petri (1923) advised grafting the sweet chestnut on to *Castanea crenata*, for the prevention of ink disease, caused by *Blepharospora cambivora* Petri. In *Citrus* culture, Dufrenoy (1925) recommended the use of Seville or bitter orange (*C. aurantium*) for the control of foot rot (*Phytophthora terrestris* Sherb.), and Nattras (1933) noted that the same species was also resistant to gummosis (*P. citrophthora* (S. and S.) Leonian), while *Citrus aurantifolia*, a species hitherto widely used as a root-stock, was extremely susceptible. An investigation of American pear stocks by Reimer (1925) showed that *Pyrus calleryana* and *P. ussuriensis* were resistant to blight (*Bacillus amylovorus* (Burr) Trev.). The latter, however, was unsuitable owing to its slow rate of growth. The commonly used French pear seedling (*Pyrus communis*), although susceptible to blight, had the advantage of being resistant to *Armillaria* root rot, and its retention for this reason was also advised by Johnston (1931). An example of a similar method in the control of insect pests, recommended by Staniland (1924), is the use of the "Northern Spy" root-stock for apples on account of its immunity to woolly aphis (*Eriosoma lanigera* Hausm.). So far, the possibility of "scion influence" affecting the immunity of the root-stocks has not been considered, but several instances in which this is believed to occur will be discussed later (pp. 278 *et seq.*).

Until quite recently, interest in the effects of grafting and budding was confined exclusively to characters of obvious economic value such as fruitfulness and its relation to vegetative vigour and development. Even here, the knowledge accumulated remained empirical, and no further progress could be made in the absence of information concerning the identity of the different stocks in use throughout the various fruit-growing regions of the world. In England, owing to the work of the East Malling and Long Ashton research stations, the classification of root-stocks is now fairly complete. The characters of the "Paradise" or vegetatively propagated apple stocks were described by Hatton (1917, 1919) in 1917-19. In the following year they were arranged into four groups, according to the relative vigour induced in each of three scion varieties: Lane's Prince Albert, Worcester Pearmain, and Bramley's Seedling (1920). A summary of the Paradise root-stock investigations was published by Pearl (1932). In all, sixteen types were recognised. An investigation of the "free" or seedling apple stocks proceeded along similar lines, and was summarised by Spinks (1931). The quince or dwarfing stocks for pears were classified in 1920, five principal types being recognised (Hatton, 1920 *a*). Results with the "free" stocks for pears, comprising six different *Pyrus* spp., were published later (1933). A botanical description of the seedling and vegetative stocks for the stone fruits was published by Hatton (1921), and the plum and cherry stocks were later investigated in detail by Hatton, Amos, and Witt (1929) and by Grubb and Witt (1925), respectively. Root-stock classification has recently attracted considerable attention on the Continent, and in Germany, for instance, Gleisberg (1930, 1931,

1931 a) has published three important summaries dealing almost entirely with the East Malling investigations. Other recent publications are those of Schindler (1932), also from Germany, and of de Angeli (1934), from Italy.

During the course of these investigations, grafting and budding were shown to affect almost every part of the plant throughout the whole course of its development. The nature of the quantitative and qualitative factors underlying stock : scion relationships has recently been discussed in detail by Hatton (1930), Gleisberg (1930), and Tukey and Brase (1933) in relation to deciduous fruit growing in England, Germany, and the United States respectively, and by Toxopeus (1931) in relation to *Citrus* culture. In particular, Swarbrick (1930) and Halma (1934) emphasised the importance of scion variety in itself modifying root-stock effect, and Hatton (1931), in a later paper, discussed the parts played respectively by the stem and root portions of the stock. The chemical basis of "stock : scion effects" was also discussed by Roach (1931).

A lack of balance between the normal nutritional requirements of stock and scion may become evident in the form of an increased susceptibility to physiological disease. For example, Hatton and Grubb (1925) showed that apples on East Malling stocks, Nos. II, V and VII, *i.e.* the "semi-dwarfing" group, were more liable to leaf scorch due to potassium deficiency than the same varieties grown on the "vigorous" stocks Nos. I and VI. Further, this could be correlated with the behaviour of the ungrafted stocks. On any one stock, however, susceptibility to leaf scorch also depended on the nature of the scion variety. As would be expected, trees on the "semi-dwarfing" stocks showed the effects of manurial deficiency sooner than those on "vigorous" (Amos *et al.* 1930), although here again this difference could be masked by the response of individual scion varieties. Similar instances of the effect of root-stock on susceptibility to nutritional disorders were reported by Hendrickson (1925) for iron chlorosis in pears, and by Perold (1927) and Ravas (1928) for lime chlorosis in the vine. Water relations may also be affected, leading particularly to various disorders in the fruit. Thus, in 1927, Heppner (1927) described a "black-end" disease of "Bartlett" pear, characterised by a blackening and hardening of the calyx end of the fruit, and occurring almost exclusively on the Japanese stock, *Pyrus serotina*. On the French stock, *P. communis*, the disease appeared only on exceptionally heavy, wet soil. The development of black-end on *P. serotina* stocks was later confirmed by Johnston (1931), and Overholser *et al.* (1933) reported that the use of this species also contributed to the development of "cork spot", a condition indicating a disturbance in the normal water supply to the fruit. Even in the absence of physiological disorders at the time of picking, the size and colour of the fruit may be affected (Rogers, 1927), or its keeping qualities impaired. According to Wallace (1932), resistance to storage breakdown in Bramley's Seedling was less on East Malling stock No. I than it was on No. V, but the fruits themselves were larger. The difference was only apparent on the manured plot. These observations were extended by Kidd and West (1934), who showed that the commercial storage life of Bramley's Seedling from trees on type IX (dwarfing), II (semi-dwarfing), I (semi-vigorous), and XVI (vigorous) was re-

spectively 20, 14, 20, and 21 weeks. The early breakdown induced by the semi-dwarfing stock, No. II, is possibly correlated with a deficiency of potash, a condition that would be expected from its high susceptibility to scorch. The effect of root-stock on infection by the numerous storage rots caused by species of *Fusarium*, *Botrytis*, *Penicillium*, etc., was investigated in a series of papers by Horne (1932, 1933, 1934). In some cases, it was found to depend entirely on the nature of the scion variety. For instance, Lane's Prince Albert on East Malling stocks X and VI gave fruit respectively highly resistant and susceptible to storage rot caused by *Cytosporina ludibunda* Sacc., though, with Bramley's Seedling, the reverse was obtained. Although, in this respect, a differential response to stock influence may be a varietal characteristic, there remains the possibility that the scion varieties themselves condition the nature of the influence exerted.

On numerous occasions, grafting and budding have given rise to abnormal conditions resembling in effect an increased susceptibility to disease, but actually due to one of the following causes: incompatibility between stock and scion, transmission of an unsuspected virus disease, or imperfect manipulation of the graft union.

The causes of incompatibility between stock and scion are uncertain, although recent work suggests that, in some cases at least, the production of "specific immunity reactions" may be a possible explanation (Neilson Jones, 1934, p. 9). Complete incompatibility is sufficient to prevent union altogether, but when partial or incomplete, it may result in a gradual reduction in growth, or in some abnormal condition observable only after a period of years. The data given by Pearl (1932) show that, in the apple, incompatibility is of little consequence except between certain varieties and the East Malling type of "vigorous" stock No. X. According to Hatton (1920 a), it exists in a more or less complete form between various pears and the quince stocks, and is also (1921; Hatton *et al.* 1929) of wide occurrence in plums. Provan (1933) has demonstrated its existence in *Citrus* spp. It has recently been described by Rathbun-Gravatt (1927) as the cause of a disorder of Japanese cherries grafted on *Prunus avium* and *P. cerasus*, and also by Chester (1930) as responsible for a "graft blight" of lilac (*Syringa vulgaris*) on *Ligustrum vulgare*. It is strongly suggested by the symptoms of a new disorder of walnuts described by Schuster and Miller (1933), although, according to Witt (1930), it has not previously been recorded in this genus. The disorder, confined to Persian walnut grafted on black walnut root-stock (*Juglans hindsii*), was characterised by a sudden girdling due apparently to the failure of the newly formed xylem tissues of stock and scion to unite. Later, the two kinds of wood became entirely separated by a dark, corky abscission layer.

Transmission by grafting, in the absence of any other causal agency, is generally assumed to establish the identity of a virus disease. This method has been used by Cayley (1932), in investigating the nature of "breaking" in tulips, and by Grieve (1934), in a comparison of the conditions known as "spraing" and "internal rust spot" of the potato. The use of grafting has also been recommended by Harris (1932) in connection with a possible virus disease of the strawberry. On the other

hand, graft transmission of a virus (or other) disease is frequently confused with other effects of grafting leading to an increased susceptibility to external agencies. For instance, Rawlins and Horne (1930) described a new "buckskin" disease of cherry to which trees on "Mazzard" stock were exceedingly susceptible, but to which those on "Mahaleb" (*Prunus Mahaleb*) were resistant. The disease resulted in the production of small, conical, shrivelled fruits, with slight chlorosis and mottling of the foliage, and was transmitted to healthy trees by top-grafting with infected scions. However, Rawlins and Parker (1934) further showed that the disease could not be transmitted through the resistant "Mahaleb" tissues. Moreover, trees on "Mahaleb" which were artificially infected rarely showed symptoms in the fruit and were severely chlorotic, presenting a strong contrast to those on "Mazzard", in which the symptoms of the fruit were predominant, with little or no chlorosis. The evidence suggests that the disease is contracted under natural conditions almost entirely by transmission from infected "Mazzard" stock. The chlorotic symptoms developed by artificial inoculation of trees on "Mahaleb" recall the "silvered" condition of Morello cherry on the same stock, observed by Grubb (1933). If the causal agency should prove to be the same in each case, a source of external infection of the buckskin disease must be looked for. A similar condition of plums, described by Atanasoff (1932) as "plum pox", is transmitted by aphids, but nothing is known of its relationship to the preceding. Atanasoff recently brought the whole question of the occurrence of virus diseases in the deciduous fruits into prominence by suggesting that bitter pit of apples was itself a virus disease, and that the prevalence of this condition in trees on the "Northern Spy" root-stock was simply the result of graft transmission from infected stock (1933). In a later paper (1934) he succeeded in showing that this theory would provide a satisfactory explanation of a number of McAlpine's (1911-16) observations. Some doubt existed at first as to the occurrence of the associated foliage symptoms, which, he considered, would prove to be homologous with the infectious mosaic of apple described by Bradford and Joley (1933), and possibly also with the condition described by Valteau (1932) a year previously. Later (1934 a), he claimed to have recognised them as a widespread, light green spotting and blotching which had previously been overlooked. On this assumption, bitter pit of apples was revealed as a member of a group of related diseases, including the infectious mosaics (*loc. cit.*), plum pox, and the buckskin disease of the cherry. Further, the "pitting" of the fruit was considered exactly comparable with the condition of "net necrosis" associated with the virus diseases of the potato.

Grafting and budding may also be responsible for the direct introduction of disease affecting both stock and scion alike. This is particularly liable to occur where the point of union is below soil level, and is generally the result of improper fitting and tying. Even if no external infection is introduced, the union between stock and scion may remain imperfect, leading to the production of "callus knots" and other abnormalities. Thus, Howarth and Chippindale (1929) described a new disease of *Rhododendron*, which they believed to originate from imperfect unions obtained by the process of saddle grafting below soil level. However, the frequent

association of certain fungal fructifications suggests that soil infection may also be involved. Soil infection during the process of grafting was also suggested by Noack (1934) as a possible cause of an obscure new lilac disease in Germany. The author noted that in Holland, where the custom was to graft 30 cm. up the stem, the disease had not been recorded. In apple and other Rosaceous hosts, malformations of the graft union are frequently confused with true "crown gall" resulting from infection by *Pseudomonas tumefaciens* (S. and T.) Duggar. The non-pathogenic developments were first distinguished by Muncie (1926), and Melhus, Muncie, and Fisk (1928) showed that they originated principally from an overhanging scion lip, left by the usual method of tongue grafting, which could be prevented by the alternative use of wedge grafts. Suit (1934) and Riker *et al.* (1929) advocated the use of various adhesive tapes and plasters to ensure a rapid and perfect union of the graft. At the same time, these methods afforded increased protection from true crown gall infection.

There remains to be considered the evidence bearing on the problem as it was defined by experimental investigations, namely, the effect of grafting on resistance and susceptibility to disease caused by pathogenic fungi and bacteria. Two possibilities have been suggested: a direct effect, due to transmission through the graft union of the substance or substances actually responsible for the reaction concerned, and an indirect effect, due to a modification of the normal response to environmental conditions.

In certain instances, the direct transmission of resistance and susceptibility by grafting appears fairly probable. Several authors have reported it in the case of crown gall of apples. Thus, Wormald and Grubb (1924) observed that the percentage of natural crown gall infection in any one stock depended on the susceptibility (on its own roots) of the scion variety with which it was grafted. This conclusion was later confirmed by Harris (1931) for artificial inoculation. A similar observation was made by Rodigin and Papaeva (1931), in the lower Volga basin, where the reaction of individual trees on the relatively susceptible "Kitayka" stock was in every case identical with that of the scion variety on its own roots, about ten varieties conferring complete resistance. Scion influence was also recorded by Oppenheimer (1926) in regard to a disease—termed "Wurzelkropf"—in some respects resembling crown gall, but for which no causal organism had been isolated. A possible explanation of these phenomena is suggested by the view that resistance to crown gall depends on the degree of acidity of the cell sap. Further examples, for which, however, no explanation can be suggested, are afforded by a group of *Citrus* diseases. Thus, Savastano (1923) recorded the transmission of root-stock resistance to wilt or "mal secco" (*Pseudomonas citriputeale* (Smith) Stapp), and Provan (1933) and Toxopeus (1931) the transmission of scion resistance respectively to collar rot and root rot gummosis (*Phytophthora citrophthora* = *Pythiacystis citrophthora* S. and S.).

In the great majority of instances, however, modifications of the normal disease relationships of stock and scion appear to result from the more subtle changes affecting their development in relation to environmental factors. The remaining data, which illustrate this point of view, and are as complete as possible, are summarised for each group of diseases, as follows:

A. Pome fruits

- (1) *Apple scab* (*Venturia inaequalis* Aderh.): *Apple mildew* (*Podosphaera leucotricha* (Ell. and Everh.) Salm.).

See Frampton (1928), Moore (1930, 1930 a, 1932), Pearl (1932). Work at East Malling has been done chiefly with the variety Cox's Orange, but also with Worcester Pearmain, Stirling Castle, and Bramley's Seedling.

Scab. Root-stock influence was found to be entirely independent of the degree of vigour induced, but was markedly affected by manurial treatment. Also, East Malling stock No. III, which was markedly susceptible on its own root, was found to induce twig and fruit resistance, respectively, in Cox's Orange and Bramley's Seedling. Nos. III, XV, and XIII, inducing resistance to scab, also induced resistance to brown rot (*Sclerotinia fructigena* Aderh. and Ruhl.).

Mildew. Root-stock influence was again entirely independent of "grouping", but was not affected by manurial treatment. Pearl (1932) mentions two examples in which resistance and susceptibility were induced, respectively, by stocks showing similar reactions on their own roots.

Hatton (1930) suggests that root-stock influence in these two diseases (apple mildew and scab) is connected with the period at which growth is commenced in spring, for which see also Swarbrick (1929).

- (2) *Canker* (*Nectria galligena* Bres.).

Apple. According to Moore (1934), root-stock effect was to some extent dependent on the nature of the scion variety. Thus, both Cox's and Stirling Castle were relatively resistant on East Malling stocks XV, VI, and I, and relatively susceptible on XVI and XIII. On the other hand, on X, and also to a lesser extent on V, Cox's was relatively susceptible, while Stirling Castle was relatively resistant. Brooks (1928) believed that immunity to canker depended on the nature of the root system of the stock. (See Rogers and Vyvyan, 1928.) However, as Swarbrick (1930) has shown, this may itself be modified under the influence of the scion.

Pear. Wormald (1927) noted that "Fertility" pear trees were more susceptible on common pear (*Pyrus communis*) than on any other stock, and suggested that this could be attributed to an increased vigour of growth.

According to Massee (1924) and Lepelley (1928) "stock : scion relationships" have not been found to affect resistance or susceptibility to apple aphid (*Aphis pomi*) or woolly aphid (*Eriosoma lanigera*).

B. Stone fruits

- (1) *Dieback*.

Neither Cayley (1923) nor Amos *et al.* (1927) were able to find any indications of stock : scion effects. Wormald (1934) considered that "dieback" of plums was largely due to bacterial canker (*Pseudomonas mors-prunorum* Worm.), and advised control by high working combined with the use of resistant stems such as common plum or Myrobalan "B".

(2) *Silver leaf* (*Stereum purpureum Pers.*).

The increased susceptibility of "Victoria" plums on Myrobalan "A", as compared with those on common plum, was noted by Brooks and Storey (1923) and also by Petherbridge (1926). In a later paper, Brooks and Brenchley (1931) showed that Myrobalan was itself the more resistant of the two stocks. In this case, the increased susceptibility was due entirely to differences in habit and to an increased brittleness of the wood.

(3) *Cherry diseases.*

Sweet cherry. Resistance to buckskin disease on Mahaleb as compared with Mazzard was confirmed by Philp (1930), who also stated that bacterial gummosis (*Bacterium cerasi* (Griffin) Elliott) was less severe on Stockton Morello.

Morello. Grubb (1933) noted slight differences in susceptibility to brown rot (*Sclerotinia laxa* Aderh. and Ruhl.) on different sweet cherry stocks. On the whole, susceptibility was greater on Mahaleb and acid stocks than on sweet cherry. A new bacterial blossom wilt was described to which distinct differences in susceptibility occurred on different sweet cherry stocks, not, however, related to vigour of growth. Mazzard and Mahaleb stocks were also compared by Greatorox (1933) in relation to cherry-growing conditions in Victoria.

IV. CONCLUSION

The possibility that grafting may lead to changes in the disease relationships of stock and scion depends on two sets of conditions, namely, the nature of the factors underlying resistance or susceptibility in each case, and the nature of the actual relationship existing between the two components. Thus, the factors responsible for a certain reaction may be transmitted directly, or the reaction may be modified or even completely reversed by changes in habit, rate of growth, or other morphological or physiological features. The best example of a direct effect of grafting is seen in the case of crown gall of apples, where resistance and susceptibility appear to be transmitted from scion to stock unaltered. Here the factor responsible is probably the degree of acidity of the cell sap, which seems to depend entirely on the nature of the scion variety. A number of cases in which grafting was shown to be without any effect on disease relationships were interpreted as evidence that resistance and susceptibility were specific properties of the protoplasm, not capable of being transmitted through the graft union, and unaffected by environmental conditions. Notably in the case of Roach's experiments with wart disease of potatoes, this conclusion has received further support from other considerations. Elsewhere, however, the possibility remains that the reaction in question was due to the presence of some relatively simple substance in the plant, which was either not transmitted at all, or else was modified by the cells which received it.

The use of inoculation experiments in the interpretation of the structure of plant chimaeras and graft hybrids was shown to depend on two assumptions, as follows:

(a) The absence of any reciprocal influence affecting the disease relationships of the two components of a chimaera.

(b) The ability of a parasitic fungus to penetrate one or two outer layers of tissue, otherwise immune to infection.

The first of these received indirect support from the negative results of the grafting experiments already referred to. Both assumptions were fully substantiated by Klebahn's results with the experimentally produced *Solanum* chimaeras, whose periclinal structure had previously been deduced from other considerations. However, in those forms whose mode of origin is not known, the evidence derived from inoculation experiments can only be suggestive. For instance, in the case of the *Crataegomespili* and *Piricydoniae*, the results with *Gymnosporangium sabinae* supported the periclinal chimaera theory, while those with *Podosphaera oxyacanthae* could just as well be used in support of the "burdo" or graft-hybrid theory. Without the assumption that the two components of a chimaera retain their characteristic reaction unaltered, there remains the possibility that they may not be distinguishable, as such, from the modified "burdo" tissues of a true graft hybrid.

The possibility of an indirect effect of grafting on disease relationships was considered by Winkler (1912), but did not recur in the experimental literature until the work of Volk (1931). In his recent book, Krenke (1933) discussed the nature of resistance in relation to the results to be expected from grafting experiments. Both "active" and "passive" immunity were recognised, of which the invariable characteristic of the former is that it consists of an actual reaction to infection, whereas the latter depends on any property which is entirely independent of infection. Both physiological and structural developments may be involved in active immunity. In addition, certain classes of resistance, not recognisable as one of the above, such as disease escaping, or resistance due to vigour of growth or regenerative capacity not in specific relationship to disease, were classed together as "growth resistance" (*Wachstumresistenz*). According to Krenke, the effect of grafting on resistance was very largely indirect, being compared with the modifications induced by changes in nutritional and other environmental factors. In fact, the more markedly specific and, strictly speaking, genotypic were the resistant properties, the less were they likely to be modified by grafting. An important distinction was made between "non-susceptibility" (*Unempfindlichkeit*), or complete genotypic inability to permit infection, and "resistance" (*Widerstandsfähigkeit*), which was a phenotypic expression capable of modification by environmental factors. Whether "non-susceptibility" could itself be "passive" in character, and depend on some property capable of transmission by grafting, was not made clear.

The value of grafting in the prevention and control of diseases in fruit trees and other species for which it is the common method of propagation, is frequently overlooked owing to the apparently greater importance of some of the other properties involved. Until recently, the lack of knowledge concerning the identity of the stocks in use in the different regions has made exact information as to their effect on disease relationships difficult to acquire. The most complete body of information concerns the use of the different East Malling apple stocks in the

control of leaf scorch and in relation to the various physiological disorders affecting quality and storage life of the fruit. A number of recommendations have been made for the use of resistant stocks in preventing soil infection in *Citrus* and in various ornamental trees and shrubs. The use of the methods of in-arching, bridge-grafting, and top-working with suitable varieties for the replacement of portions of the tree lost or damaged by disease or mechanical injury is well known. Many apparent instances of an increased susceptibility to infection induced by grafting can be attributed to incompatibility between stock and scion, or occasionally to the presence of an unsuspected virus disease. Again, malformations resulting from imperfect manipulation of the graft union have frequently been confused with crown gall tumours. Where the union is below soil level, extra precautions are necessary to avoid infection by external agencies. The use of grafting in the control of mildew, scab, canker, and other common diseases of deciduous fruits is confined, in most cases, to a limited number of varieties only, very few generally applicable recommendations having been made.

V. SUMMARY

Disease relationships in grafted plants and chimaeras are shown to have both theoretical and practical significance.

Grafting experiments have been employed as a means of investigating the nature of resistance and susceptibility to diseases caused by pathogenic fungi and bacteria. The effects of grafting may be direct, owing to transmission through the graft union of the substance or substances actually responsible for the reaction concerned, or indirect, due to a change in the normal response to environmental conditions. Negative results, while necessarily inconclusive, indicate that resistance and susceptibility are either genotypic properties of the protoplasm, or else are due to some factor that is not, as such, transmissible.

Inoculation experiments have also been used in the interpretation of graft hybrids and chimaeras. In the case of the artificially induced *Solanum* chimaeras, experiments with *Septoria lycopersici* have shown that the two components retain their characteristic reaction to infection unaltered. Unless this assumption can be made in other forms, whose mode of origin is unknown, it becomes impossible to distinguish the two components, as such, from the modified tissues of a true graft hybrid. Results with the *Crataegomespili* and *Pirocydoniae* are not altogether consistent with the periclinal chimaera theory.

Examples are given of the practical importance of grafting in the prevention and control of disease and mechanical injury in fruit trees and other ornamental trees and shrubs. Choice of suitable stocks may entirely prevent leaf scorch and other physiological disorders, and a large body of information has accumulated concerning the influence of root-stock on quality and storage life of the fruit.

The importance of incompatibility between stock and scion is discussed, and examples are quoted in which grafting has led to the transmission of an unsuspected virus disease. Improper fitting and tying of the graft union is liable to result in the production of wound overgrowths, and also increases the danger of external infection.

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THE SIGNIFICANCE OF THE CHLORIDES IN TISSUES AND ANIMALS

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I. GENERAL SIGNIFICANCE OF CHLORINE

THE element chlorine is a common component of organisms and of their aqueous environment. It is particularly abundant in the sea, where it comprises nearly 2 per cent. of the total solution and 55 per cent. of the dissolved material. Sea water forms the environment of many organisms, and there are some peculiarly interesting relations between the salts of sea water and those which are contained in organisms. The salts of the sea are usually regarded as the accumulated results of the steady inflow of very dilute salts brought by the rivers. For ages the river salts have been concentrated by evaporation, and the sea water of the present day is the result. But the sea salts are quite different in composition from the river salts, the difference in chloride content being particularly conspicuous. The average composition of salts flowing into the ocean includes only 5.68 per cent. chlorine (Clarke, 1920), while the ocean salts contain nearly 10 times as large a proportion. It is evident that the formation of ocean salts is the result of a process of concentration which has selectively increased the proportion of chlorine while it has selectively eliminated other constituents.

The selective elimination applies particularly to the calcium of natural waters. Calcium carbonate is deposited in the sedimentary rocks, which contain practically no chlorine. The precipitation of calcium carbonate in the sedimentary rocks has been mainly accomplished by living organisms, and in this respect life has been a factor in determining the present composition of the sea. Is it then profitable to consider whether the failure of chlorine precipitation to occur in natural waters is related to the failure of organisms to include chlorine among their insoluble compounds? The question suggests facts regarding the composition of organisms which are more significant than an attempt to secure a final answer.

For many reasons the study of the general physiological significance of chlorine is best founded upon our knowledge of the composition of mammals. Chlorine daily passes through the mammal in rather large amounts. The reserves of chlorine are small and can be rapidly exhausted. A few hours of sweating and copious water drinking, or the loss of gastric secretion during a few days by persistent vomiting or diarrhoea, produce serious consequences on account of the loss of chlorides. In fact, so prompt is the injurious effect of an increased loss of tissue chlorides that it is doubtful whether there exists a true reserve. It seems rather that the composition and environment of the cells require the presence of a constant concentration of chloride, but that none of this chloride is permanently situated; it is constantly circulating through the solid tissues as well as through the circulating fluids of the body.

From the standpoint of its chemical properties, the possible biological uses of chlorine appear to be limited. Chlorine does not enter oxidation-reduction systems, nor does it form natural organic compounds, and chloride ions have comparatively little specific influence upon tissues. For the present discussion we will regard the lack of specificity of chloride ions as the basis for the significance of the chlorides, and we will consider the chloride ions as relatively indifferent complements in maintaining the ionic balance of the tissues.

Eventually we may distinguish the component phases of tissues. At present we can only analyse whole tissues. Nevertheless it is striking that a given tissue like muscle or blood is so constant in composition throughout the animal kingdom, for organisms show a surprising lack of originality in composition and metabolism. We have therefore selected our data on the basis of the amount of chlorine found in a unit weight of tissue. It is rarely profitable to attempt to describe the individual cell from an analysis of a tissue, for the cell is not a quotient obtained by dividing a physiological reaction by the number of participating cells.

II. THE STATE OF THE TISSUE CHLORIDES

Chlorine, neutral chlorides, and organic chlorides are quite different, and it is important to know the state of the element in the tissues. For analysis the organic matter is first destroyed by ashing, and the chlorine is precipitated as insoluble silver chloride. In principle and practice the method is easy to apply, but certain precautions must be observed for consistently accurate results (Cullen, Wilkins

and Harrison, 1933; Sunderman and Williams, 1933). By this procedure no information is given concerning the original condition of the element in the cells. But there are several indications that it is dissolved and ionised like the chloride in sodium chloride. The evidence is perhaps indirect, but nevertheless very extensive. The careful and numerous analyses of mammalian plasma, red blood corpuscles and tissue fluids, show that the chlorine enters membrane equilibria as if it were derived from the equivalent amount of sodium or potassium chloride (Peters and Van Slyke, 1931, Chap. xix). In studies of osmotic phenomena of blood, tissues, and cells, whether by the method of freezing-point depression, vapour pressure (Hill, 1930), or by observing water distribution, the osmotic effect of tissue chlorine analytically observed is equivalent to the effect derived from the presence of the same amount of sodium chloride. In fact, the dissociation of the tissue chlorides is supported by most of the criteria which support the theory of electrolytic dissociation.

It has been reported that from 10 to 50 per cent. of the chlorine in tissues exists as organic chlorides (Hanke and Donovan, 1927), but the evidence has not been developed. Peters and Man (1934) found about 4 per cent. of the chloride of sera to be present in some non-ionised form with the lipoid fraction. It has frequently been proposed that the secretion of strong hydrochloric acid by the gastric mucosa is accomplished by the extrusion from the cells of such an organic chloride, which decomposes and forms free hydrochloric acid only in the lumina of the secreting glands. The possibility was first expressed by Claude Bernard (1859, p. 371) in the form of a question, the answer to which he refused to consider for lack of evidence. But the idea is frequently proposed to explain why the cells are not themselves digested by the strong acid which they secrete (Harvey and Bensley, 1912). However, the existence of such an organic chloride has not yet been demonstrated, and no analogous neutral chloride is known which could spontaneously decompose to form strong hydrochloric acid (Irving and Wilson, 1932).

On the whole the evidence for the existence of non-ionised chlorine in the tissues is rare and uncertain. The evidence that neutral chlorides alone occur, while not final, is certainly extensive and impressive. Hence the figures in Table I and subsequently will be taken to represent the concentration of chloride ions in each tissue.

III. THE CHLORIDE CONTENT OF VARIOUS ADULT TISSUES

For the remainder of this discussion chlorides will be considered to exist only as salts of alkali metals. The prevalence of chloride in large amounts in all tissues is indicated in Table I. These figures are the most complete analyses available of the tissues of any one kind of animal and compare favourably with shorter investigations made by Cullen, Wilkins and Harrison (1933) and Sunderman and Williams (1933), who have been particularly interested in developing accurate methods for chloride determinations. Considerable variation exists in results obtained by different investigators, but discrepancies also occur in a series of

analyses on the same tissue by one worker. However, all figures present a characteristic order of magnitude for the chloride content of each tissue in the body. The reproductive organs, lungs and kidneys have the highest chloride content, glands are intermediate, while muscles, nerves and bones, have the lowest chloride content of any tissues in the body.

Table I. *Chloride content of mammalian tissues (mg. per 100 gm. fresh tissue)*

Animal ... No. of subjects ... Investigator ...	Man 1 Magnus- Levy (1910)	Dog 2 Damien (1921)	Cow 1 Vladesco (1925)	Dog 6 Cameron and Walton (1928)	Rat 9 Cameron and Walton (1928)	Man 108* Close (1933)	Rat 3 Winter (1934)	Average in mg. per 100 gm.
Ovaries	—	—	—	190	290	—	—	240
Uterus	—	—	—	201	—	270†	—	235
Lung	260	240	244	230	196	220	200	227
Testes	226	214	—	187	222	—	214	213
Kidney	208	224	225	251	178	190	197	210
Thyroid	169	—	—	161	—	180	—	170
Spleen	161	179	153	171	134	170	135	158
Pancreas	161	—	—	138	—	—	—	158
Cartilage	—	—	—	190	—	130	—	152
Salivary glands	133	—	184	152	125	—	—	148
Liver	—	117	89	136	132	150	108	122
Bone	—	—	—	103	125	110	138	119
Heart	124	136	102	119	111	130	86	115
Brain	131	22	—	148	108	173	99	114
Plain muscle	61	—	—	—	84	160	151	114
Skeletal muscle	61	72	50	67	60	80	43	62

* Although 108 autopsy subjects were used, the number of determinations on single tissues ranged from 6 to 97.

† From Kochmann and Krüger (1926).

Investigators point out the difficulty in these analyses of freeing a tissue from blood and connective tissue. Blood contains 360 mg. of chloride per 100 c.c., and connective tissue may contain large amounts of chloride. These two factors undoubtedly influence the position of a tissue in the above series. The iron content (Magnus-Levy, 1910) may be used as an indication of contamination with blood. The human spleen and lungs have 72 and 67 mg. per cent. of iron respectively, and hence in part they owe their position in the series to the blood present. That the positions of the other tissues are not appreciably affected by the inclusion of blood is shown in the low iron contents of the kidney and testicle which are 16 and 5 mg. per cent. respectively.

The water content of tissues varies from 71 to 85 per cent. (Close, 1933), but in spite of this fact the figures for chloride retain the above order when expressed in milliequivalents per kg. of tissue water. This is seen in the following figures from Close (1933): the human kidney contains 64.8, nerve 61.3, heart muscle 45.6 and voluntary muscle 29.4 m.eq. of chloride per kg. of tissue water.

IV. THE MOVEMENT OF CHLORIDES

The fact that each tissue of the mammal contains a characteristic concentration of chloride is indicated in Table I. A characteristic concentration implies constancy, but does not necessarily signify that the same chloride remains fixed in a tissue. In fact, as we shall point out, the situation is rather that chloride ions are continually shifting about from one system to another.

In the dog about 0.123 per cent. of the body weight is chloride (Rosemann, 1910, 1911*a*), while according to Magnus-Levy (1910) a 70 kg. man contains 85 gm. of chloride or 0.121 per cent. Adolph (1930*a*), in his tabulation of the proportions of the exchange of various substances in man, calculates that 2.76 per cent. of the total body chloride is excreted each day, but that this is normally replenished by the ingestion of a similar amount, so that the total body chloride of an individual is unaltered by excretory processes.

Table II. *Chloride in human secretions*

Author	Secretion	Volume	Cl %	Cl
Rosemann, G.*	Saliva	1000 c.c. per day	0.2	2 gm.
Starling (1933, p. 570)	Gastric juice	3000 c.c. per day	0.5	15 gm. per day
Bodansky (1930, p. 166)	Hepatic bile	900 c.c. per day	0.3 (dog)†	3 gm. per day
Gamble and McIver (1928)	Pancreatic juice	700 c.c. per day	0.3	2 gm. per day
Starling (1933, pp. 960 and 974)	Urine	1500 c.c. per day	0.6 (average)	6-10 gm. per day
Peters and Van Slyke (1931)	—	1500 c.c. per day	1.2 (max.)	Up to 24 gm. per day (p. 767)
Fishberg and Bierman (1932)	Sweat	4020 c.c. in 3 hours	0.3 (max.)	12 gm. in 3 hours
Moss (1923-4)	Sweat	—	0.2 (max.)	7.2 gm. in 3 hours

* From Bethe *et al.* (1927, 3, 821).

† The concentration of Cl in human bile was assumed to equal that found by Reinhold and Wilson (1934) for dogs.

The quantities of chloride that may be utilised in the daily formation of secretions are indicated in Table II. Of these, only sweat and urine eliminate chloride from the body. The kidneys may excrete as much as 24 gm. in 1 day (Peters and Van Slyke, 1931, p. 767), but the amount excreted is closely regulated to fit the daily intake without effecting depletion. Sweat formation occurs in the regulation of body temperature and differs from urine in not being subservient to the control of water and electrolyte balances. The largest definite figures that we have encountered for the chloride loss in sweat are those of Moss (1923-4), who found 7.2 gm. excreted in the course of 3 hours, and Fishberg and Bierman (1932), who obtained 12 gm. in 3 hours. In these short periods about 10 per cent. of the body chloride was eliminated. The depletion of chlorides by sweating may be serious if water is simultaneously consumed in quantities sufficient to dilute the remaining body chlorides, and in that case the condition of "miners'" or "stokers' cramps" ensues. The addition of sodium chloride to the drinking water removes

or prevents these symptoms (Moss, 1923-4). Since both sodium and chlorine are lost in equivalent quantities, it is not possible to ascribe the cramps to loss of chloride alone, or to distinguish whether the condition may not be due simply to dilution with water and consequent water poisoning.

Of the body secretions which are ordinarily not lost, the secretion of the parietal cells of the gastric mucosa is particularly interesting because it contains only chloride and hydrogen ions without other cations (Hollander, 1934). If gastric juice is lost, only chloride is removed from the electrolyte reserves, since the hydrogen ions are obtained from water. In this case then any resulting symptoms are due specifically to chloride depletion. Earlier workers found it difficult to reduce the chloride reserves by the removal of gastric juice. Cahn (1886) was unable to affect the chloride reserves in dogs by feeding them on a diet low in chloride and daily washing out their stomachs. He reports that "der Organismus hält seinen für ihn unentbehrlichen Material hartnäckig fest". Rosemann (1911*b*), by diverting the entire gastric secretion of dogs through gastric fistulae, could only reduce the normal chloride content by 20 per cent., and the concentration in the blood was not appreciably altered. However, by daily washing out the stomachs of dogs with intestinal obstruction, MacCallum *et al.* (1920) were able to bring about severe depletion of body chloride which was indicated in the diminished blood chloride and increased bicarbonate, similar to the results of intestinal obstruction. In dogs with gastric fistulae Dragstedt and Ellis (1930) observed the daily elimination of about one-sixth of the total chloride of the animals. The loss per day was greater than the normal amount present in the blood. Secretion of hydrochloric acid continued until the blood chloride was only one-third of its normal value.

It is interesting that gastric secretion will continue to the point of producing fatal deficiency of chloride, and the inadequacy of the so-called chloride reserves is shown by the rapid onset of serious symptoms. Administration of chloride ions in the form of sodium chloride was immediately beneficial to MacCallum's (1920) dogs. Dragstedt and Ellis (1930) reported that intravenous injections of sodium chloride not only caused a speedy recovery, but preserved for months animals which otherwise would have perished within a week. Strikingly beneficial effects of the administration of sodium chloride alone are now conspicuous in the treatment of a variety of diseases. Unfortunately, it is not usually clear whether the benefit is derived from restoration of the salt balance, the proportion of water, or the supply of a single ion. The problem can only be approached by careful analyses of the solid tissues, and the data are still too scanty to be of general significance.

The secretory processes listed in Table II might be instrumental in the movement of about 32 gm. of chloride per day, which is about a third of the total body content. These figures, however, do not indicate the whole extent of the movement of chloride. For example, in the formation of urine there must pass across the glomerular membranes daily 55 times as much chloride as is eventually eliminated (Cushny, 1926), or 550 gm. in this case. The transport of carbon dioxide involves a transfer across the membrane of the red blood corpuscle of 178 gm. of chloride per day (Jacobs, 1931). Hence the total chloride moved from system to system in

the body is much greater than Table II signifies. The transport of chloride may be accompanied by an equivalent movement of base as it is in the formation of urine, or chloride ions may be exchanged for other anions. The situation in this latter respect is only clear in two of the examples to which reference has been made—the secretion of hydrochloric acid by the stomach, and the exchange of chloride for bicarbonate through the red blood cell walls.

The composition of the tissues has been described as if the chloride concentration were constant and characteristic for each tissue, and the available analyses justify such a view. Under these conditions can there be any true reserves or depots? The analytical evidence indicates that skin and connective tissue are relatively rich in chlorides (Rosemann, 1911*a*; Adolph, 1930*b*; Magnus-Levy, 1910), but Rosemann and Magnus-Levy point out that sampling those tissues is particularly difficult, and that the variation in results may be as easily attributed to the lack of uniformity in sampling as to the action of the tissues as reserves.

The indirect evidence for the existence of such depots seems very inconclusive. Rosemann (1911*a*) fed a dog with large amounts of sodium chloride, and at death found that chloride constituted 0.163 per cent. of the body weight compared with the normal average of 0.112 per cent. When a man or dog absorbs or excretes excessive amounts of sodium chloride, only a small change can be observed in the blood chloride.

The disappearance from dog's blood of intravenously injected chloride is extremely rapid—more rapid even than the disappearance of the water which is injected. Both liver and muscle take up some of the chloride (Adolph, Gerbasi and Lepore, 1934). If relatively large amounts of sodium chloride are intravenously introduced into cats, the vapour pressure of the blood remains for several hours at an elevation corresponding to uniform distribution of all of the extra salt through the entire water of the tissues (Hetherington, 1931). On account of analytical difficulties it is as yet impossible to demonstrate that the extra chloride is uniformly distributed, but there is certainly no evidence that any particular tissue acts as a special reservoir for chloride.

V. THE COMPOSITION OF ISOTONIC TISSUES AND FLUIDS

We have been impressed with the extensive movement of the chloride ion, either accompanied by its equivalent of base or in exchange for other anions. Peters and Van Slyke (1931, Chap. XIX) have discussed the latter type in some detail in relation to a variety of pathological conditions, and have pointed out that, in order to keep the plasma reaction constant, the chloride ions play a role complementary to that of the anions produced in the metabolic disturbance. An examination of the chloride content of various tissues (Table I) and fluids (Table VI) of the mammalian body has shown that, although all contain some chloride, the amount varies greatly from system to system, and seems always to be inversely proportional to the anion which characterises the system. This fact, along with studies on embryos which will be considered later, suggested that, even in the

original development of specialised systems from an undifferentiated mass, the chloride ion played a complementary part, and that the final content in the adult body is the resultant of changes in other anions. The fact that chlorides occur in each tissue and fluid in an amount which is easily determined is a matter of convenience, since its determination gives the initial indicator of the make-up of the system. If two tissues of the same organism possess different chloride concentrations the remaining anionic compositions must differ. If the chloride concentration changes, one knows that a complementary alteration in other anions has occurred.

(1) *The constancy of the total base concentration*

In a discussion of anionic exchanges between systems the assumption is inherent that the total anion concentration in each system is constant. Now, in the dissociation of salts in any aqueous solution one of the most useful postulates is that equal numbers of positively and negatively charged ions are formed. Hence, according to the law of electroneutrality, the concentration of anions must be equal to the concentration of cations. If the cation concentrations in the tissues or fluids between which anion exchanges are made can be proved to be the same and constant, the original assumption is justified.

Table III. *Total base in human serum (m.eq. per litre)*

Investigator	Method	No. of subjects	Range of figures	Average
Kramer and Tisdall (1922)	Sum of cations	10	?	158.5
Briggs (1923)	"	2	146.6 - 158.3	153.6
Salvesen and Linder (1924)	"	7	147.3 - 158.5	153.3
Stadie and Ross (1925)	Total base	?	162.5 - 165.5*	163.7
Peters, Bulger, etc. (1926)	"	15	147.2 - 161.3	155.7
Sunderman, Austin, etc. (1926)	"	8	150 - 157.6	154.7
Van Slyke, Hiller, etc. (1927)	"	?	146.6 - 148.2†	147.5
Salvesen (1928)	Sum of cations	12	149.12 - 160.55	153.96
Darrow and Hartman (1929)	Total base	11	145 - 159	150.7
Peters, Wakeman, etc. (1929)	"	11	152.0 - 158.0	153.9
Stander, etc. (1929)	"	3	151.9 - 159.4	153.3
Atchley and Benedict (1930)	"	10	148.5 - 156.7	151.9
Hoffman (1933)	"	15	152.7 - 158.6	155.3
Hald (1934)	"	10	142.0 - 153.1	146.5
Peters and Man (1934)	"	5	142.0 - 151.2	145.6

* Three figures are quoted.

† Three "total base" methods were compared. Four figures are quoted.

The cations which are experimentally determined are those surviving ashing, *i.e.* sodium, potassium, calcium, magnesium, and, to the sum of these, the term "total base" is usually applied. It should be pointed out that certain organic bases could exist in the form of salts at the pH of the body, but the few analyses available indicate that their concentration in tissues is too small to be significant. The fact that no other constant is so tenaciously guarded as the total base of the serum (Peters and Van Slyke, 1931, pp. 761-4) has been accepted, especially since the first clear statement of that proposition in the work of Gamble, Ross and Tisdall (1923). The figures available in the literature for total base in human sera

are collected in Table III. Although diverse methods were used, a surprising agreement exists between the results of fifteen groups of investigators. The average value is 153.2 m.eq. per litre of serum with a range of 142.0-165.5. This range is somewhat larger than that usually quoted for the absolute variation in sera of normal humans. While the methods of determining single cations are difficult, the determination of total base is particularly liable to error. We feel that the lower values in the more recent work, especially those of Hald (1934) and Peters and Man (1934), are the most acceptable.

The sera of other mammals (Table IV) contain approximately the same amount of total base as human serum. The values for rat and horse sera more nearly approach those for human, while figures for dog and cat sera are consistently higher, by about 10 m.eq. per litre. Also the limits between which all figures fall are more widely separated, being from 144 to 195, and even the averages range from 148.0 to 178.7. We can only conclude from direct analyses that the total base of serum of all mammals is the same within fairly wide limits, 142-195 m.eq. per litre, with an average value of 153 for humans and 160 for other mammals. The large range may be attributable to methods which are still inadequate, rather than to the fact that serum total base varies to this extent.

Table IV. *Total base in animal serum (m.eq. per litre of serum)*

Investigator	Animal	No. of subjects	Method	Range of figures	Average
Bulger, Allen, etc. (1928)	Dog	5	Total base	158.3-195.0	177.2
Gamble and McIver (1928)	"	2	"	159 -175	167
Ball (1930a)	"	6	Sum of cations	144 -155	149
Loeb, Atchley, etc. (1933)	"	3	Total base	153.5-162.3	156.5
Reinhold and Wilson (1934)	"	6	Sum of cations	?	160.7
Darrow and Cary (1934)	"	2	"	P.E. of mean ± 1.46	159.16
Baumann and Kurland (1926)	Cat	11	"	166.2-185.2	175.0
Swingle and Eisenman (1927)	"	11	"	146.0-186.4	162.8
Zwemer and Sullivan (1934)	"	18	"	—	178.7
Shohl, Brown, etc. (1931)	Rat	8	Total base	?	148.5
Heller and Paul (1934)	"	?	Sum of cations	?	167.9
Smith and Smith (1934)	"	13 samples	"	—	148.0
Smith and Smith (1934)	"	10 samples	Total base	P.E. of mean ± 1.23	148.1
Van Slyke, Wu, etc. (1923)	Horse	4	"	143.4-154.8	149.0

Since the body fluids are secreted from the blood, and since tissues are bathed in serum exudates, it is convenient to compare their total base contents with that of serum. The water content in these systems varies from 75 to 98 per cent., and, because we are considering the osmotically active substances, it is only proper to compare figures expressed in milliequivalents per litre of tissue water. These are stated for solid tissues in Table V, and for serum and other body fluids in Table VI. Table V is a more or less complete list of the available data, while Tables VI and VII contain only the best complete analyses. The figures represent the averages of only the determinations performed by each investigator on normal subjects.

Table VI seems to indicate that the pancreatic juice of the dog contains less cations, the hepatic bile more, and the gastric juice an amount equivalent to the total base in serum. It was established by Gilman and Cowgill (1931, 1933) that the osmotic pressure of the gastric juice was controlled by that of the blood serum, and their observation that chloride was the only osmotically active anion was further substantiated by Hollander's demonstration (1934) that pure parietal juice contained no other cation except hydrogen. Hence the chloride and hydrogen ions of this gastric juice are each equivalent in amount to the total base of the serum. The situations with regard to the pancreatic juice and hepatic bile are not so clear-cut. It is probable that the figures quoted here for pancreatic juice are too low, because in experiments where the total bases in the juice and serum were determined simultaneously (Ball, 1930a), no discrepancy was observed. Also, many experiments indicated that the osmotic pressures of the two were equal. The total base of hepatic bile, however, is variable and seems to be consistently higher than that in serum, whereas its osmotic pressure is constant and the same as that of serum. We are accustomed to consider that the total base content gives a measure of the osmotically active substances present in a system. This may be incorrect, and the suggestions of Reinhold and Wilson (1934) that some of the taurocholic acid in bile has aggregated and become osmotically inactive, and of Hammarsten (1924) that some of the base has been rendered inactive by binding with taurocholic acid, are worthy of consideration.

Table V. *Total base and water content of solid tissues*

Investigator	Subject	Tissue	Water content %	Total base in m.eq. per litre tissue water		Method
				Range of figures	Average	
Katz (1896)	Frog	Muscle	81.6	—	159	Sum of cations
Meigs and Ryan (1912)	"	"	79.9	—	190	"
Fenn (1934)	"	"	80	—	180	"
Fenn (1934)	"	Nerve	75	—	185	"
Hill and Kupalov (1930)	Mammals	Muscle	76	—	214	"
Katz (1896)	Rabbit	"	76.8	—	200	"
Matsumoto (1933)	"	Brain (white)	69.42	215-228	229	"
Matsumoto (1933)	"	" (gray)	81.85	179-214	200	"
Katz (1896)	Man	Muscle	72.53	—	190	"
Cullen, Wilkins, etc. (1933)	"	"	—	84-215	165	Total base
Cullen, Wilkins, etc. (1933)	"	Right ventricle	—	118-219	156	"
Cullen, Wilkins, etc. (1933)	"	Left ventricle	—	131-239	163	"
Cullen, Wilkins, etc. (1933)	"	Liver	—	122-220	172	"
Cullen, Wilkins, etc. (1933)	"	Kidney	—	97-240	169	"
Bodansky (compiled by Hari) (1930, p. 497)	"	Brain (white)	68-73	—	214	Sum of cations

In spite of the somewhat greater variation which exists in the total base contents of different mammalian tissues (Table V), the average for all tissues together is 187 m.eq. per litre of tissue water, which is not much greater than the average in serum. It is improbable that the osmotic pressure of tissue cells differs from that

of serum or that the content of electrolytes is materially different. The range of the results obtained by Cullen, Wilkins and Harrison (1933) lead us again to the conclusion that the variation is the result of inaccurate methods. On the other hand, we should bear in mind that the excess of base in solid tissues over the amount in serum may indicate the existence of ionised but osmotically inactive anions. Our own experience has assured us that not only are the total base methods in their present form difficult to apply to serum and body fluids, but they are totally inadequate for solid tissues. Methods for the determinations of single cations are somewhat more reliable, but few figures are available.

We can only conclude from this review that the total base concentrations of mammalian fluids and tissues are of the same order and, although actual analyses show a wide range of variation, the concentrations probably approach the value in serum of 160–190 m.eq. per litre tissue water. Since we are concerned with differences in anions (see Table I) which are for the most part greater than the range of variation in total base, we shall consider that the total base and therefore the total anion concentration are for the purposes of our argument approximately constant.

(2) *The anionic composition*

The chloride content of serum is even more constant throughout the several types of mammals represented in Table VI than the total base. It is the most abundant anion and binds some 70 per cent. of the base, the remainder being provided for by bicarbonate, phosphate and serum protein anions. The digestive juices, although initially derived from the blood serum, contain greatly divergent amounts of chloride. Pancreatic juice has on the whole less chloride and more bicarbonate than serum, but the results are variable. Ball (1930*b*) showed that the faster the juice flowed the higher the bicarbonate content, and in consequence the lower the chloride. The flow of juice had previously been abnormally stimulated by secretin until Johnston and Ball (1930) prepared dogs with pancreatic ducts permanently open to the exterior. The analysis of this juice formed without artificial stimulation gave lower bicarbonate values than formerly and the chloride contents ranged from 88 to 138 m.eq. per litre of juice. Hepatic bile contains a specific type of ion, the taurocholate anion. Its presence causes a lower chloride content than that in serum, but again a reciprocal relation exists between the amount of taurocholate and the sum of the chloride and bicarbonate anions. The chloride of gastric juice is equivalent in amount to the total base of serum, thereby excluding all other anions. The mechanism, whereby chloride is secreted into the gastric juice and bicarbonate into the pancreatic juice in excess of that in serum, is still a matter of conjecture.

A graphic presentation of the anionic make-up of the serum, digestive juices and solid tissues of different organisms is given in Figs. 1 and 2. To facilitate comparison the anions are expressed as percentages of the total base which was analytically found for the system in question.

Chloride is present in the solid tissues (Table I) in even more diverse amounts than in the body fluids, the range being from 62 mg. per 100 gm. of fresh tissue in skeletal muscle to 240 mg. in ovaries. What anions, then, replace the chloride deficits

Table VI. *Composition of mammalian body fluids (m.eq. per kg. tissue water)*

Investigator	Subject	No. of subjects	Total cations	Cl'	HCO ₃ '	HPO ₄ ''	Protein'	Bile acid anions	Total determined anions
1. Serum (water content 92 %)									
Peters and Van Slyke (1931, p. 762)	Human	?	168.5	111.9	30.4	2.2	17.4	—	161.9
Hald (1934)	"	10	159.2	112.0	29.4	2.5	18.0	—	161.9
Bulger, Allen, etc. (1928)	Dog	5	192.6	123.1	24.8	3.7	11.3	—	162.9
Loeb, Atchley, etc. (1933)	"	3	170.1	116.5	25.1	—	15.5	—	—
Reinhold and Wilson (1934)	"	6	174.7	119.6	23.9	—	—	—	—
Baumann and Kurland (1926)	Cat	11	190.0	127.2	—	3.0	—	—	—
Zwerner and Sullivan (1934)	"	18-21	194.2	129.9	17.7	3.0	—	—	—
Smith and Smith (1934)	Rat	—	160.9	111.1	24.1	2.6	22.0*	—	159.8
2. Pancreatic juice (water content 98 %)									
Schmidt (1854)	Dog	—	167	56.2	—	0.61	—	—	—
Hartmann and Elman (1927)	"	—	147	51.0	60.2	0.13	—	—	111.3
Gamble and McIver (1928)	"	—	164	82.7	80.6†	1.02	—	—	—
Johnston and Ball (1930)	"	2	156	132.7	22.5	0.4	—	—	155.6
3. Hepatic bile (water content 93 %)									
Ravdin, Johnston, etc. (1932)	Dog	4	196.9	90.5	51.6	—	—	38.4	180.5
Reinhold and Wilson (1934)	"	6	207.3	68.9	36.6	—	—	81.7	187.2
4. Gastric juice									
Gilman and Cowgill (1933)	Dog	5	—	156	—	—	—	—	156
Hollander (1934)	"	5	170	170	—	—	—	—	170
Wilhelmj, etc. (1934)	"	5	—	163	—	—	—	—	163

* Calculated from percentage protein using Cohn's (1925) value, i.e. 1 gm. serum albumin at pH 7 binds 35×10^{-6} gm. equivalent of alkali.

† Not determined, but obtained by difference.

Table VII. *Electrolyte composition of solid tissue (m.eq. per kg. tissue water)*

Author	Subject	Tissue	Total cations	Cl'	HCO ₃ '	HPO ₄ ''	Lactate'	Total determined anions
Hill and Kupalov (1930)	Mammals	Muscle	214	23	16	151	2	192
Katz (1896)	Rabbit	"	200	18.8	—	85	—	—
Hill and Kupalov (1930)	Frog	"	182	19	7	95	3	124
Fenn (1934)	"	"	180	18	11	91	3	123
Matsumoto (1933)	Rabbit	Brain (white)	229	67.3	—	20.9	—	—
Matsumoto (1933)	"	Brain (gray)	200	62.7	—	25.8	—	—
Fenn (1934)	Frog	Nerve	185	61	15	24	11	111
Manery and Irving (1935a)	<i>F. heteroclitus</i>	One day after fertilisation	346	247	1	40	—	288
		At hatching	346	188	8	80	—	276

in certain tissues? Complete analyses of solid tissues are relatively few in number. However, those presented in Table VII will serve to illustrate the tenor of our discussion. Skeletal muscle, both frog and mammalian, seems to be characterised by a relatively high concentration of base-binding phosphate fractions, since they

bind about 50 per cent. of the entire base. The anions supplied by muscle proteins are not directly determined, but are conventionally considered to be equal in amount to the difference between the total base and total determined anions, and it is probable from such a calculation that they bind about 30 per cent. of the base.

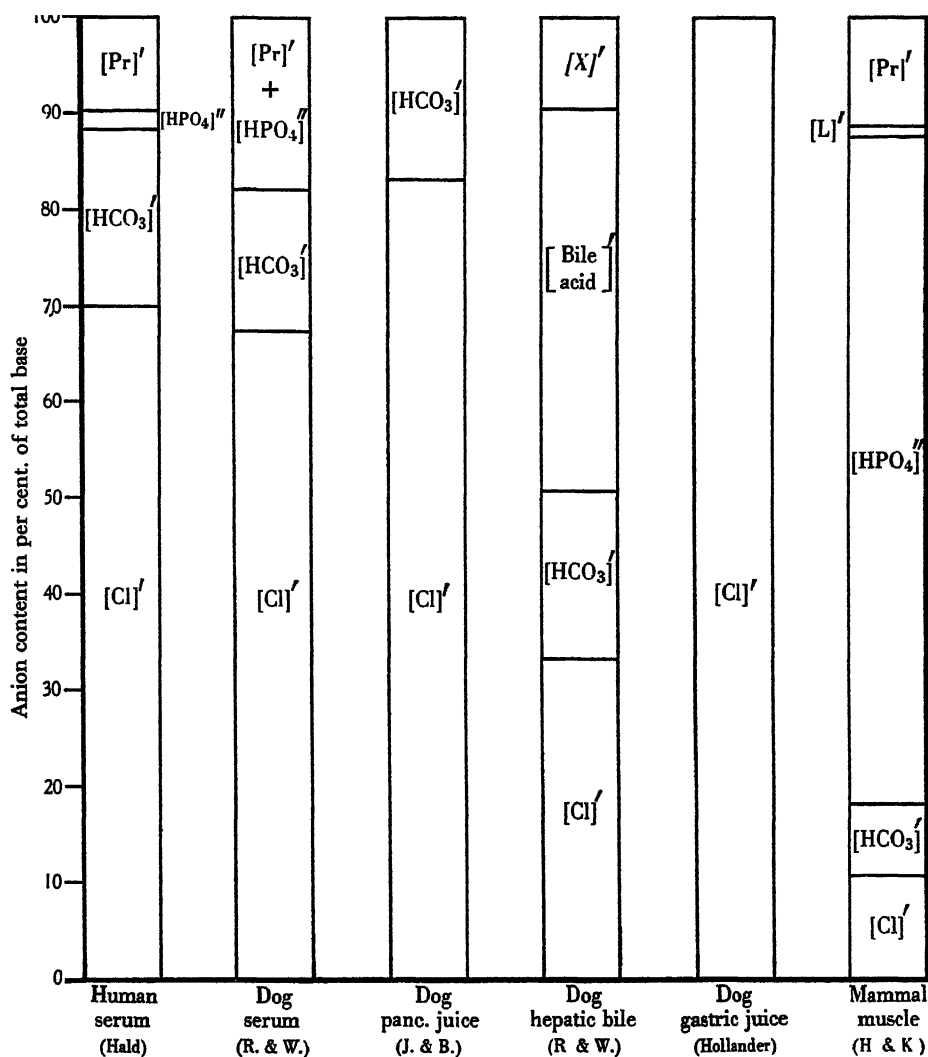


Fig. 1. The anionic composition of tissues and fluids of some warm-blooded animals. Bracketed letters refer to authors in Tables VI and VII.

The remainder is distributed between chloride and bicarbonate with the exception of 2 or 3 m.eq., which in resting muscle may be bound by lactate ions. The anions in nervous tissue are of the same character as in muscle, but they differ in distribution. Phosphate anions bind only about 10 per cent. of the total base, chloride 30 per cent., bicarbonate the same, and lactate ions slightly more base than

in muscle. There remains, then, some 50 per cent. to be bound by the anions of nerve proteins.

An examination of Figs. 1 and 2 will demonstrate the complementary role of chlorides by clarifying the following points: that all parts of an organism contain chloride in amounts greater or less than that in serum; that each is more or less characterised by the predominance of a specific anion, bile juice by taurocholate, muscle by phosphate, nervous tissue by protein, etc.; that the amount of chloride always bears a reciprocal relation to the quantity of the specific ion present; that in gastric juice chloride is the specific anion.

VI. IONIC CHANGES IN EMBRYONIC DEVELOPMENT

At first sight it does not seem that the anionic composition of tissues indicates how the existing differences between tissues have developed, or how they are maintained. To postulate a selectively permeable membrane acting like a barrier is only making another morphological assumption. Membrane equilibria describe, in part, the permeability of the red blood corpuscle, but the idea of a membrane equilibrium is inapplicable to a process of active secretion. The development of secretions or the maintenance of differences in ionic composition of tissues actually represents lack of equilibrium and the expenditure of energy.

(1) *The decrease in chloride concentration*

To understand how adult tissues with different electrolyte compositions have developed, chemical studies should be made on the mass of undifferentiated embryonic cells which is the common source of all adult tissues, and should be followed until an organised body is formed. Indications have appeared that the chloride concentration changes in a characteristic way with development.

In 1873 the proposition was made by Bunge that the chloride concentration of the young mammal decreased as the animal developed. In the following year he published figures which showed that this occurred in the rabbit (Table VIII). No further advance was made until 1910 when Rosemann (1910, 1911c), by relating all of the available data to his own, demonstrated that the chloride concentrations of the cat, the dog and the human were reduced during their early development (Table VIII). Needham's (1931, p. 1282) graphic relation of the data on the chloride in the human foetus clearly indicated its decrease with age. Recently Iob and Swanson (1934) made a fairly complete study of the chemical changes in human foetuses from the ages of 4-10 lunar months. A gradual fall in the chloride concentration took place during this period (Table VIII) which could not be due to dilution by water, since the water content likewise fell from 89 at the 4th to 75.5 per cent. at the 10th month. Other organisms exhibiting a similar decrease in chloride concentration with development are presented in Table VIII. Needham (1935) has referred to the similar change in chloride concentration in the chick embryo found by Yamada (1933), as being negatively heterogonic. Since the chloride content of an organism increases less rapidly than its weight, it is probably appropriate to say

that growth is negatively heterogonic with respect to chloride (Needham, 1934¹), although most of the data are not sufficiently precise to permit rigid application of a formula.

Table VIII. *Chloride changes during early development*

Author	Animal	Age or wt.	Cl in mg. per 100 gm. wet wt.
Bunge (1874)	Rabbit	Embryo (19 gm.)	208
		14 days (105 gm.)	135
Rosemann (1910)*	Cat	New-born (162 gm.)	208
		19 days (182 gm.)	197
		Adult (2350 gm.)	159
	Dog	4 days (354 gm.)	231
		Adult (9945 gm.)	105
	Man	Foetus (248 gm.)	272
		" (841 gm.)	223
		" (1339 gm.)	221
		New-born (2821 gm.)	176
Magnus-Levy (1910)	Man	Adult (—)	121
Iob and Swanson (1934)	Man	4.3 lunar months	273
		6.4 "	255
		8.2 "	227
		10.0 "	183
Winter (1934)	Rat	1 hour after birth	245
		7 days "	182
		17 days "	150
Mankin (1930)	Chick	4th day of incubation	375
		10th day "	200
		20th day "	113
Irving and Manery (1934)	Speckled trout eggs	1st day after fertilisation	143
		53rd day "	102
Manery and Irving (1935a)	<i>F. hetero- clitus</i> eggs	1st day "	706
		11th day "	554

* These figures are Rosemann's compilation of his own data and those of earlier investigators.

Of all the animals referred to in Table VIII none is so well adapted to a study of this kind as the teleost egg, which is an entity of convenient size containing an undifferentiated mass of yolk at fertilisation and a well-developed fish at hatching. In the case of the speckled trout (Irving and Manery, 1934) and *Fundulus* (Manery and Irving, 1935a), the early egg was found to contain considerable chloride which steadily decreased as the fish body was organised. This was not just an apparent loss, because the water content did not vary after the initial intake at laying occurred (Manery and Irving, 1935b). After these considerations it seems reasonable to suggest that chloride reduction is a characteristic accompaniment of embryonic development. It may indeed continue until an adult body is formed. Its occurrence has been recorded only for the early states because the rapidity of growth in this period produces changes large enough to be determined analytically.

(2) *The formation of new anions*

A loss of anions must be balanced by other ion changes in order to maintain electroneutrality. In the case of the teleost egg an actual excretion of chloride to the exterior must occur, which may be accompanied by an equivalent amount of base. However, conductivity measurements (Gray, 1919), determinations of the depression of the freezing-point (Svetlov, 1929) and of ash content (Hayes, 1930) of various teleost eggs indicate a constant electrolyte concentration during development, and a loss of neutral chloride is therefore improbable. Some preliminary determinations of the total base content of developing *Fundulus* eggs (Table VII) corroborated this view (Manery and Irving, 1935*a*). Although the variation in total base was large, it was less than the change in chloride content.

Since the chloride lost is not accompanied by base we are confronted with the problem of finding new anions in compensation. The trout egg presents a unique system. Its development in a fresh-water environment, from which it is isolated, precludes the absorption of new anions from the exterior, and therefore one must look to intrinsic metabolic processes for the production of anions. The evolution of carbon dioxide in respiration is characteristic of most living cells, and so bicarbonate ions are first to be considered. Bicarbonate anions definitely increased in teleost eggs as the embryo body developed, but in speckled trout eggs the gain in bicarbonate was only one-seventh of the chloride loss (Irving and Manery, 1934), and only one-eighth in the case of *Fundulus* eggs (Table VII, and Manery, Warbritton and Irving, 1933), and thus full compensation for chloride loss was not effected by the increase in bicarbonate concentration.

Yolk which makes up the bulk of the early egg is rich in phospholipins, which are incapable of binding base (Jukes, 1935). An embryonic body, the greater part of which is functioning muscle, must contain inorganic phosphorus and the ionising organic phosphates which characterise muscle. These bind as much as 2 m.eq. of base per mg. of phosphorus (Hill and Kupalov, 1930). Plimmer and Scott (1909) reported that phospholipins decreased in the developing chick egg in favour of inorganic and acid-soluble phosphate. Rosenheim *et al.* (1928) and H. Schmidt (unpublished) detected a similar occurrence in brown trout and *Fundulus* eggs respectively. Using our own values for total phosphorus, which does not vary during development of *Fundulus* eggs (Manery and Irving, 1935*a*), those of Schmidt for ether-soluble phosphorus, and the distribution of phosphorus anions which occurs in muscle (Hill and Kupalov, 1930), we have arrived at the calculations presented in Table VII and Fig. 2. These figures and calculations are arbitrary, but serve to illustrate that new base-binding phosphate fractions are undoubtedly incorporated in the body of the fish at the expense of phospholipins.

Finally, we have shown that the "net" pH of the trout egg becomes more alkaline during development (Irving and Manery, 1934). A protein on the alkaline side of its isoelectric point binds base, and the more remote the pH of the environment is from the protein isoelectric point the higher the base-binding capacity of the protein. Hence the mere shift in pH concurrent with development from

fertilisation to hatching results in the formation of new protein anions. Hayes (1930) also found that new and different proteins appeared as salmon eggs developed. If these should possess a more acid isoelectric point than the proteins initially present, then the increased dissociation of protein would provide new

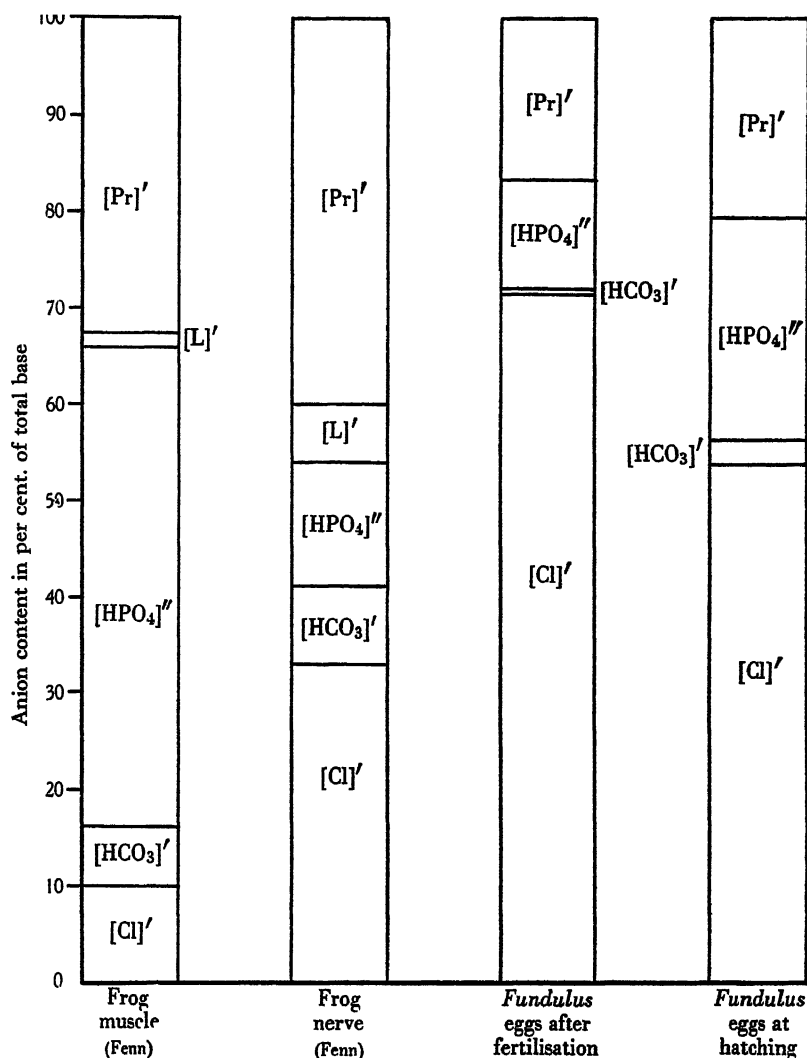


Fig. 2. The anionic composition of frog tissues and developing *Fundulus* eggs.

anions. Calculations show that the bicarbonate changes which are known, together with the phosphate and protein anions which are in all probability made available during growth, compensate the entire chloride deficit.

During the early development of teleost eggs, therefore, the cation concentration does not vary but chloride anions are lost, and new bicarbonate, phosphate and

protein anions are gained. Only the anions undergo changes in concentration. It is worth noting that since the cation concentration is constant, the metabolism which results in the organisation of a body proceeds principally through the anion-forming substances. The process may be briefly described. Chloride is initially present in the undifferentiated mass of yolk and binds a large proportion of the cations which will later become the total base element of the embryonic body. As growth proceeds and new anions are formed to compose the embryo body, an equivalent amount of the base is released for them by the excretion of chloride.

The possibilities of this scheme for describing ionic changes during development have only been worked out in the case of the eggs of the trout and *Fundulus*. However, the known reduction in the chloride content of developing mammalian foetuses, as well as the probable changes in the phosphates, suggest that the scheme may be usefully applied as a guide for analytical studies of ionic changes during embryonic development. It has been previously noted that chloride ions are relatively inert, and in adult tissues generally play roles which are complementary to other anions (section V (2)). The behaviour of chloride ions as inert complements is exemplified in a much more clear-cut manner in embryonic development than in any of the other instances which have been cited. Chloride loss in favour of new metabolic anions is proposed as an important fundamental characteristic of embryonic development.

VII. CHLORIDE CONCENTRATION AND SPECIALISATION OF TISSUES

It has been shown that the unspecialised mass of tissue which exists early in development is characterised by a high chloride content. As development proceeds the tissue becomes differentiated and highly specialised organs are formed. Concurrent with specialisation the chloride concentration of the embryo as a whole decreases. The decrease in chloride content with specialisation is also exhibited in Table I. Here adult mammalian tissues are arranged in order of their chloride concentrations (see section III). The reproductive organs, such as the ovaries, testis and uterus, have the most chloride, the glandular and secretory tissues less, and brain and muscle contain least of all. The organs concerned with reproduction are considered to be the most primitive and least specialised in the body. Glandular tissues which are more specific in function are, however, much less specialised than bone, brain and muscle. Skeletal muscle which is restricted to a single and unusual type of activity contains only one-quarter of the chloride found in ovaries. Even among the muscle tissues themselves chloride decreases with specialisation. Heart and plain muscle which are more primitive in type than skeletal muscle possess twice as much chloride. This survey of the chloride concentration of adult tissues shows that specialisation corresponds with the degree of reduction in chloride concentration. The similar situation which exists in many of the digestive juices of the adult mammalian body has been discussed in detail in section V (2). Juices which contain a specific anion such as the taurocholate ion in bile juice are characterised by a low chloride concentration. The fact that the tissues and fluids

making up an organised body had a common source in the undifferentiated embryonic cells will be used later to provide us with a clue to the origin of the differences in their ionic make-up. Some significance may be attached to the fact that the early mammalian embryo contains chloride (Table VIII) in approximately the same concentration as the unspecialised reproductive organs (Table I).

It is apparent that the processes of growth and specialisation are accompanied by a reduction in chloride concentration. Low chloride concentration corresponds with a high degree of specialisation, and, because it is easily determined, it is useful as the initial step in studying the electrolyte composition of tissues and fluids.

Attention should be drawn again to the fact that only the tissue as a whole has been considered (section I), and that no differentiation has been made between the tissue cells and the intercellular spaces. Fenn, Cobb and Marsh (1934) and Fenn (1934) have advanced evidence that the chloride of muscle and nerve tissues is chiefly present in the intercellular spaces. This view should furnish a better description of the changes and differences in cells than the facts which we have described on the basis of the analyses of complete tissues. At present Fenn's view demonstrates principally the dependence of the function of the muscle and nerve cells upon their immediate environment.

VIII. THE MECHANISM UNDERLYING CHLORIDE REDUCTION IN GROWTH AND SPECIALISATION

The excretion of chloride from the teleost egg during its development bears such implications and is of such magnitude that some discussion of the mechanism underlying the process should be attempted. The common theories of membrane equilibria cannot provide an adequate description of the situation. Furthermore, there are many similar places in the adult mammalian body where theories of membrane equilibria cannot explain ionic movements. The formation of glandular secretions, and especially the gastric juice, does not conform to membrane equilibrium conditions (Hollander, 1934). The fact remains that these movements of ions do occur, and that they occur only in living and active cells. Dr B. P. Babkin suggested to us that the cells of the gastric mucosa and of the developing embryo possess the same common function of excreting chlorides, and we have presented evidence (section IV) that chloride ions move across cell boundaries more freely than any other anions. Evidence is rapidly being accumulated that glandular secretion is dependent on oxidative processes, and that it cannot be described in terms such as filtration, osmotic pressure, diffusion or membrane properties. Höber's view (1934) that an "active physiological permeability" existed in glandular cells implied the ability of the cell to drive materials against a concentration gradient as well as the fact that energy was consumed in the process. Recently Visscher and Ingraham (1935) have obtained some evidence that salt absorption from the gut is linked up with oxidative reactions. Ionic shifts in various plant cells seem also to depend on the energy derived from their respiratory metabolism.

In the embryonic development exemplified by teleost eggs a separation of ions occurs, which must involve a utilisation of energy. What are the exothermic reactions which might support this process? An examination of the changes concurrent with the excretion of chloride brings forth possible sources of energy. Carbon dioxide is evolved as the end-product of respiratory metabolism, and bicarbonate becomes a permanent component of the developing organism. The metabolism of phospholipins and the resulting formation of phosphate fractions is an important process accompanying growth. The pH shift toward alkalinity indicates a change in hydrogen and hydroxyl ions. Bicarbonate, phosphate and hydrogen ions are produced by the exothermic reactions in metabolism. The source of energy for the transport of chloride ions undoubtedly lies in these metabolic processes. The formation of new bicarbonate, phosphate and protein anions which are incorporated in the developing organism requires a compensatory change in other anions to maintain electroneutrality. Chloride ions, being relatively inert, do not participate directly in oxidative processes, and their excretion is merely a resultant of the reactions concerned with growth.

Although this discussion has been principally based upon changes in developing teleost eggs, it is probably applicable to embryonic growth in general. It can also be used to describe many situations in the adult organism where the separation of ions is brought about by an expenditure of energy. In many body fluids and specialised tissues the chloride is decreased in favour of a specific anion, and chloride exists in concentrations differing greatly from that in blood. It is not conceivable that the formation and maintenance of these secretions and tissues are divorced from metabolic processes. We would emphasise the fact that most of the ion shifts occurring in a growing or in an adult organism cannot be defined as equilibrium states or by the properties of membranes. The movements of ions are essentially the results of the application of energy from the exothermic reactions of metabolism. As such, they would not approach equilibrium during life, unless one could postulate an at present fantastic equilibrium in which vital processes were included.

It is difficult to estimate the concentration of individual organic anions by any direct method, and anions vary in kind in different tissues and fluids. Chloride, however, occurs in every tissue and fluid in amounts which can be conveniently determined. Since the analytical method is comparatively simple it is desirable to start a description with the chloride content of the tissue. We know that the total anionic composition is approximately constant, and hence that any change in chloride entails a complementary change in other anions. Chloride content may be thus used as a clue to electrolyte changes in metabolism. Also, if two tissues of the same organism possess different chloride contents, then, the remaining anionic compositions must differ. These considerations, along with the view that a low chloride concentration is a sign of high specialisation, makes chloride analysis a useful tool for biologists.

IX. SUMMARY

1. Chlorine is an important component of an adult animal organism as a whole, since its depletion quickly results in serious consequences.
2. Chloride occurs in large and characteristic amounts in all tissues and fluids. Although a constant net composition is maintained, this chloride is not fixed, but an extensive movement occurs daily between different systems of the body. No conclusive demonstration of large chloride reserves has as yet been made.
3. A study of teleost eggs showed that the total base concentration remained constant, that during development the chloride concentration was reduced, and that new bicarbonate, phosphate and protein anions compensated for the chloride lost. Growth proceeded through changes in anions rather than changes in cations. Chloride reduction is a characteristic of all embryonic growth.
4. That chloride reduction accompanies specialisation is shown in embryonic development. Also the chloride concentration of adult tissues and fluids is inversely proportional to their degree of specialisation.
5. The loss of chloride with growth cannot be explained by theories of membrane equilibria. The energy necessary for the excretion of chloride is derived from metabolic processes involving bicarbonate and phosphate anions. Chloride ions do not participate in oxidative reactions but behave as relatively inert complements to other anions.
6. Because chloride is so easily determined, and since its movement attends changes in the concentration of other anions, and since low chloride is a sign of high specialisation, its concentration in tissues and fluids is useful as an initial indicator of the electrolyte make-up or an electrolyte change in a system.

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THE TRANSFORMATION OF ORGANIC DESIGNS: A REVIEW OF THE ORIGIN AND DEPLOYMENT OF THE EARLIER VERTEBRATES

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I. INTRODUCTION

THE teleology of Paley, checked for a time by Darwin's exposition of the nature of adaptation, was long since quietly readopted in essentials by the greater part of our race. Under a changed name teleology has captivated even major prophets of the new science. One dates himself therefore as a Darwinian when one maintains that the appearance of conscious fore-ordination of natural designs is illusory.

An organic design might be defined as a collocation of parts of an organic whole varying in magnitude, emphasis or distance in time or space from some point of origin or reference. Under this definition the song of the peacock is as much an organic design as the pattern of his tail coverts, and to the same general class of phenomena we may refer the behaviour patterns of social insect states and those of *Homo sapiens* in a given period and country.

A teleologist might well object, however, that such a definition really begs the question, by omitting the criterion of usefulness to the possessor. But deferring for the moment the question "What is usefulness?" we may readily admit that innumerable organic designs strongly resemble humanly designed mechanisms or

bear an uncanny suggestion of purposive arrangement of values. Paley did well to stress the wonder of the mechanism of the human ear, and nothing can be gained, it seems, by minimising or ignoring the lock-and-key relationship of organic designs with their physical and biotic environment.

In dealing with such grand themes as Adaptation, the easiest way is to fall back on agnostic or obscurantist generalities, such as that the essential nature of life must, almost by definition, for ever remain unknown to a race of beings who can look at life only from the viewpoint of an ephemeral creature, conditioned in a thousand ways by a limited environment. History shows that it is but a short step from this agnosticism to the classic "why-might-not" and "what-if" schools of wishful escape. On the other hand, experience suggests that there is a great deal in the idea that man has become a "time-binding" animal, who by a sort of triangulation from many viewpoints is somehow managing to gain an increasingly fair representation of the cosmos as a whole. In short, science advances by surmounting the barriers set for it by its own mystics. It will indeed be shown in outline below that since the time of Darwin, Huxley and Haeckel considerable progress has been made in tracing the major adaptations during the deployment of the vertebrate classes and that some of the general ways in which organic designs have undergone evolutionary changes may now be formulated in surprisingly simple terms.

The primary sources of the material summarised in this article are the hundreds of fossil and recent skeletons and parts of skeletons of vertebrates of all classes which it has been my privilege to study, often with the aid of graduate students of Columbia University. Our studies, however, have constantly been guided and illuminated by the discoveries made by a host of palaeontologists and morphologists, many of whose names appear in the references to literature at the end of the article.

II. NATURE AND ORIGIN OF ORGANIC DESIGNS

(1) *Factors of organic designs*

Carbon dioxide, water and other common materials of the terrestrial environment have remarkable properties that are indispensable for the maintenance of life, as set forth by Henderson and others. From the teleological viewpoint these and other elements, although perhaps occurring separately elsewhere, were assembled on this terrestrial ball in the right proportions to afford an adaptive basis for living protoplasm. An enquiry into the origin of the adaptive reactions of protoplasm lies beyond the scope of the present article, but granting the fitness of the environment, the energy-giving properties of solar radiation in reaction with a labile protoplasm and the fundamental properties of variability and heredity, it is certain that geologic time has been long enough for divergence, change of function and the elaboration of organic designs. The history of the deployment of the vertebrates presently to be summarised appears indeed to afford direct evidence in support of Darwin's view that organic designs have arisen through the accumu-

lation and integration of small changes continued through long stretches of geologic time.

(2) *Evolution of parts of organic designs*

"Distrust the man with the simple formula" might well be the first rule of safety for all cautious men. Yet in the face of it I am suggesting that all changes in organic designs, no matter how complex, have arisen through the interaction of two ideally simple processes—repetition and emphasis; in other words, that repetition and emphasis finally result in transformation. Of course either factor may vary from zero upward and either may precede the other in time, any particular organic design being the resultant and residuum of all the changes since the assumed starting-point.

The very beginnings of scientific ideas or discoveries of wide scope are often notoriously elusive, so that at least the germs of the evolutionary principle which I have called *polyisomerism*¹ (repetition) and *anisomerism*¹ (emphasis) (*Proc. nat. Acad. Sci.*, Wash., 20, No. 1, January 1934) may perhaps be shown to have been anticipated in part by Democritus, author of the atomic theory, by Herbert Spencer (*Principles of Biology*, 1867, especially p. 215) and other philosophers; in any event I am sure that parts of this principle were clearly apprehended by Cope (1871), T. H. Huxley (1880), Dohrn (1876), Bateson (1894), Osborn (1929), J. S. Huxley (1932) and others.

It would defeat the purpose of the present article to go into a lengthy historical digression at this point, and it may be sufficient to touch here and there upon the history of the discovery of the role of repetition and emphasis in the evolution of organic designs. In 1871 Prof. E. D. Cope, one of the most versatile and prolific naturalists of the nineteenth century, read before the American Association for the Advancement of Science a paper "On the method of creation of organic forms", in which he attempted to explain the origin of various structures and organs among the vertebrates by invoking, first, a principle which he called "antero-posterior repetitive acceleration", and second, the principle of "lateral repetition, the result of a repetitive effort of growth force in a transverse direction". This is a clear anticipation by more than half a century of my own belated invention of the terms "polyisomerism" and "polyisomerism", to denote respectively the products of repetition and the process itself. In 1880 T. H. Huxley (p. 650) noted that the evolution of the feet in the family of the horses had involved increase of certain parts, reduction of others and fusion of those that remain. This is a principle stressed by Williston (1914, pp. 3, 21, 22) as operating in the evolution of the skull of vertebrates. I have called this principle "Williston's law", but it is a special case of my "anisomerism", involving either the uneven development (positive or negative) of certain polyisomerismes, with or without fusion, or the selective emphasis of growth forces within varying limits or times.

Prof. Henry Fairfield Osborn in various papers has dealt with "rectigradations" (= "aristogenes"), which are by definition predetermined by hereditary potential,

¹ Defined in Summary, p. 339 below.

and "alloiometrons" or changing dimensions, which likewise by definition are entirely free of hereditary control in allied phyla. Polyisomeres, on the other hand, are merely the results of organic reduplication, however caused, and anisomeres originate either in ontogeny or in phylogeny from the uneven development or reduction of polyisomeres. Even the song of a bird is composed of polyisomeres and anisomeres, and the same is true of all organic designs. In short, structural polyisomeres involve reduplication at nearly uniform rates, while anisomeres result from local accelerations and retardations, appearing first in variable degrees in the individual development but gradually becoming emphasised in phylogeny.

Of course with regard to particular patterns we seldom know exactly why rates of growth either vary or remain constant at certain focal points. Herbert Spencer argued (1867, pp. 119-65) that many changes in the forms of plants are due to response to changes in the incidence of gravitation, food and the like. No doubt modern botanists and zoologists have made progress in separating environmental from hereditary forces, but in that direction it is not now necessary to go.

In order to illustrate further the application of the terms polyisomerism and anisomerism and to show their bearing on the general problem of how organic designs are changed, let us consider briefly the history of the hypurals or bones that support the fleshy peduncle of the tail in typical teleost fishes. This history has been well established through the labours of many palaeichthyologists, especially Traquair and A. S. Woodward. In *Cheirolepis*, the oldest known ganoid fish from the Old Red Sandstone, the tail is heterocercal, much like that of a sturgeon (Fig. 1 A), that is, the rear end of the notochord is prolonged into the dorsal portion of the tail, which bends upward slightly, so that the dermal web or sweep of the tail is directed downward and backward. This dermal sweep is strengthened internally by short rods extending beneath the vertebral column and doubtless serving as attachment points for the primitive tail muscles, which are derived from the axial segmental muscles or myomeres.

At the other end of the series in a perfected tail of the tarpon type we find that the tail muscles are transformed into large and complex anisomeres which bear little resemblance to the simple segmental muscles from which they were derived. They are supported by equally large hypural bones (Fig. 1 C) derived by anisomeric emphasis and fusion from some of the simple hypural rods of the ancestral fishes.

In between these extremes (Fig. 1 B) lie many intermediate conditions illustrated in various fishes of the orders Chondrostei, Holostei, Amioidi, Isospondyli. Thus the highly efficient organic design of the percoid tail has been derived by anisomerism or emphasis of certain of the simple polyisomeres of the ancestral stage.

Use and usefulness have no doubt been conditioning factors in the selective process, so that for our present purpose we do not have to make any awkward choice between the Darwinian and Lamarckian explanations. But it will be observed that in the case of the evolution of the homocercal from the primitive heterocercal pattern, there has been a change of function on the part of certain axial muscles, which became hypertrophied and differentiated so as to impart new and special motions to both the peduncle and the web of the tail. What is true of

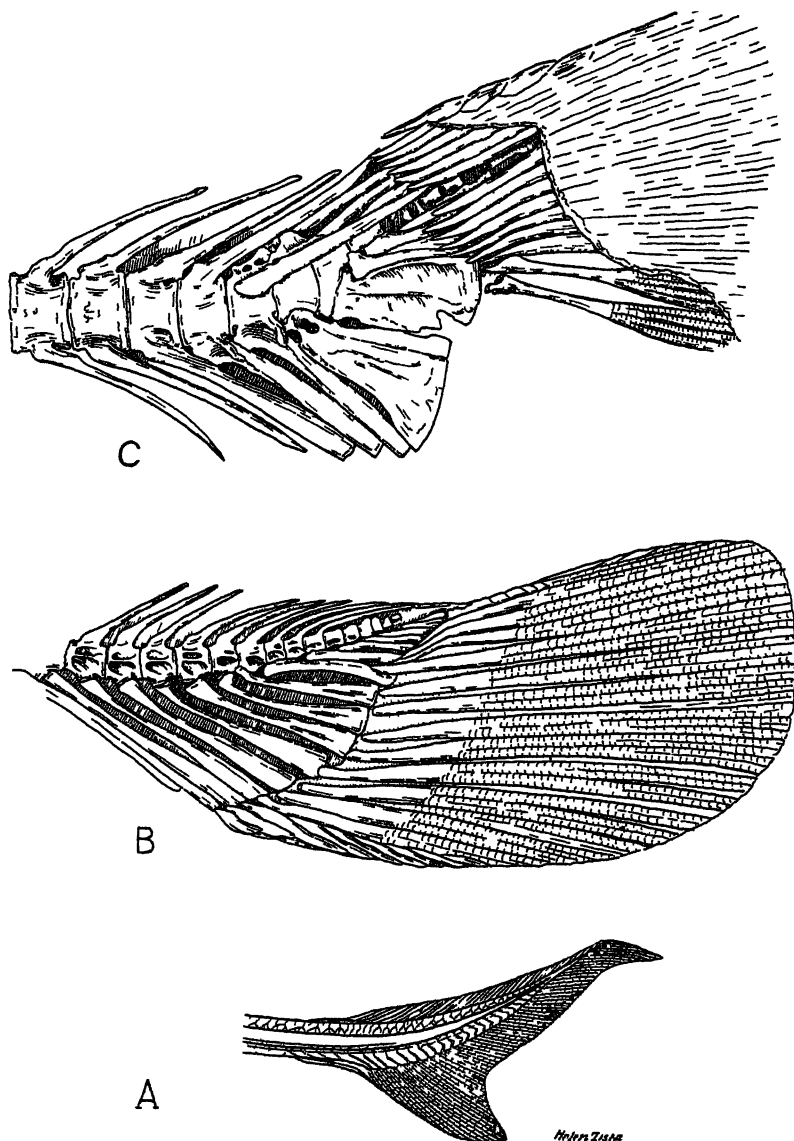


Fig. 1. The evolution of an organic design: changing relations of polyisomeres and anisomeres illustrated in three structurally successive stages of the tail bones of fishes. A. The hypural bones appear as simple polyisomeres. Primitive chondrosteian ganoid stage, represented by a modern sturgeon (*Acipenser sturio*), a "living fossil" of early Palaeozoic ancestry. B. Some of the polyisomeres are becoming somewhat larger than their fellows (incipient anisomerism). Intermediate holostean ganoid stage, represented by a modern garpike (*Lepidosteus*), a survivor of Mesozoic ancestry. C. Advanced anisomerism, involving reduction in number, with great enlargement and partial fusion of certain units. Typical teleost stage, represented by the tarpon, a survivor of early Caenozoic ancestry.

the foregoing example may be shown to be true in thousands of like cases. Thus the parts of organic designs change through reduplication (polyisomerism) and differentiation (positive or negative anisomerism).

As a concomitant of these changes any given part after long geologic time finds itself in somewhat changed relations of space, time, function and value to the adjacent parts, to the organism as a whole, and to the parts of its environment. Finally it should be noted that polyisomerism and anisomerism do not always alternate; both may and frequently do operate during the same period in producing patterns of considerable intricacy.

(3) *Evolution of organic designs as wholes*

Fortunately in the present enquiry we do not have to decide between the older view that changes in structure follow changes in habits, and the newer view (Cuénot, Davenport) that changes in structure determine changes in habits. namely, that after each "preadaptation" in either physiological reaction or in structure the race will exploit its new opportunities; as in the case of an increased hereditary tolerance for fresh water or the possession of longer jaws or larger spines, and the like. In either case the interactions between changing habits and changing functions might perhaps be visualised as serrated curves with nearly coincident serrations.

After a long time, through the cumulative effect of successive movements of polyisomerism and anisomerism, the individual will come to differ widely and in many features from his remote ancestors. At such a time, providing we have fairly adequate material for comparing the individual with his ancestor of a given geologic age, we are ready to distribute his anatomical make-up under two main headings: (1) *Habitus*, or the totality of characters relating to present habits of feeding, locomotion, reproduction and the like; (2) *Heritage*, or the totality of characters surviving from much earlier ages and gained in adjustment to earlier environments.

As an example, we may say of ichthyosaurs that their general habitus was fish-like but their heritage was distinctly reptilian. But part of the habitus of the remote ancestor becomes part of the heritage of the descendant; we therefore observe that habitus and heritage are correlative terms, like father, son, and grandson. Hence in analysing the successive stages of evolution of organic designs, we may often usefully qualify the words habitus and heritage so as to indicate their source or relative age. For example, the generic habitus of the "electric eel", *Electrophorus*, is unique, since this fish possesses an electric organ of peculiar power and construction, but its somewhat eel-like form is a family heritage (Gymnotidae); its subordinal heritage is that of the characins (Heterognathi), while its more ancient ordinal heritage (the possession of the Weberian ossicles) is that of the Ostariophysi (characins, carps, catfishes), and was in earlier ages doubtless a new habitus character that appeared in some single family of isospondylous heritage.

Again, the habitus of the South American "ostrich" (*Rhea*) is to some extent ostrich-like, but certain heritage characters seem to ally it with the carinate ancestors of the tinamous.

Changes in habitus and heritage, like changes in individual parts, are brought about by the accumulation of polyisomerism and by the modifications resulting in anisomerism. Consequently polyisomerism and anisomerism are the ways in which divergence, parallelism and convergence of phyletic lines take place. If the polyisomerism and anisomerism proceed at leisurely paces and are not too unevenly distributed over a structural pattern, the resulting changes, especially during short periods, will be recognised as examples of what I have called *undeviating evolution* in preference to orthogenesis, rectigradation and the like. But if the rates of change are rapid and inconstant, if they are emphasised and concentrated during embryonic and foetal stages, or if they are continued over very long periods, the results may fairly be classified as examples of *transformation*.

Unfortunately in mammalian palaeontology the later histories of many groups, which are known only over relatively short times, appear to be cases of undeviating evolution. From this circumstance, as well as from the sustained and successful efforts of modern palaeontologists to distinguish more and more lines of ancestry, there is now arising an apparent tendency to regard mammalian evolution as the result solely of orthogenetic or, as I prefer it, undeviating evolution; but if we consider the evolution of mammals as a whole and contrast such an excessively specialised form as a whalebone whale with its terrestrial placental ancestors, which were doubtless primitive swift-running carnivores, we cannot fail to realise that in the skeleton as well as in many other parts of the anatomy there has been a profound transformation in which but little of the original design is left.

To those palaeontologists, zoologists and others who have not happened to be vitally interested in the problems of the origin and deployment of the vertebrate classes, the title alone of the present article may be enough to condemn it. For it must be admitted that the age-long differences in viewpoint and objectives between the adherents of *l'école des faits* and those of *l'école des idées* are no less acute now than they were among the French and German naturalists of the eighteenth century (Gregory, 1910, p. 88). The particularists, often the authors of impeccable catalogues of facts, demand more and more carloads of complete fossil skeletons and love nothing so much as to enjoin caution and condemn theories. The generalisers, in reconstructing the history of the past, can only study the shards and fragments with the vision and enthusiasm of Egyptologists; holding fast to Hutton's and Lyell's demonstration of the persistence of geologic forces and processes, they must likewise recognise the reality of the *échelle des êtres* in every age and learn to appreciate the numerous gradations between the ultra-conservatives on the right, the liberals in the centre and the radicals and freaks on the extreme left. Although the title of the present article might suggest a strong bias towards the school of ideas, at the present time there does not seem to be any compelling reason why either one's facts or one's ideas should be altogether inadequate, especially in view of the enormous accessions of new and better material that have enabled the recent authors named below to advance many of the major problems of vertebrate evolution from the stage of speculation to the stage of accepted results.

III. ORIGIN OF THE VERTEBRATES

Unfortunately we are confronted at the beginning of the present section by a great uncertainty regarding the invertebrate ancestors of the vertebrates, which persists in spite of a century of attack by zoologists and palaeontologists. This uncertainty rests partly upon the well-known fact that the main phyla of present-day invertebrates were already distinct from each other in the lowest Cambrian deposits at the beginning of the Palaeozoic era, and that fossil remains from earlier ages are extremely scarce and limited, so far as known, to very low forms of life (Walcott). The vertebrates alone of the great phyla of animals remain undiscovered in the Cambrian rocks, but in the next period, the Ordovician, they are already represented by fragments of the first known ostracoderms, or pre-fishes.

Nevertheless, some progress has been made by the method of elimination, which disposes of the annelids, the nemerteans, and all branches of the jointed or arthropod groups as possible ancestors of the vertebrates. With regard to the annelids, the phylogenetic speculations of Semper, Dohrn, Minot and many others, although much too summarily disposed of by Delage and Hérourard (1898, pp. 346-8), all rest on assumed homologies between the several parts of the anatomy of annelids and of vertebrates; but these alleged homologies cannot be admitted as valid: first, because the basic designs of these two phyla are so profoundly different that a mere general correspondence in either position or function between particular parts in annelids and vertebrates cannot be accepted as evidence of relationship; secondly, because no alleged intermediates between the two phyla have ever been proved to be such.

The same general objections are fatal to the theories of Gaskell (1896) and of Patten (1912), which set forth mutually destructive sets of equations of assumed homologies between the several parts of arachnoids and of vertebrates. The structures which Patten labels "notochord", "mesencephalon", "first vagus neuromere", and so forth in arachnids have never been admitted by other morphologists to be homologous with the corresponding structures in vertebrates; moreover, the brain and spinal nervous system of the ostracoderms, as reconstructed from remarkably full evidence by Stensiö (1927), differ so widely from those of arachnids that "homologies" between them can only be far-fetched and arbitrary.

On the contrary, it seems far more probable that such general and superficial resemblances as do exist between primitive arachnoids and primitive vertebrates are an expression of convergent evolution. In view of the prevalence of tendencies toward reduplication of segments in many lines of animals, it would not be surprising if similar sets of polyisomeres had been selected in both phyla as the basis for improvements in the locomotor apparatus. Subsequently by parallel anisomerism some of the neuromeres became larger and partly fused to secure better co-ordination of movements. Thus the basis of Patten's group Syncephalata, including the arthropods and the vertebrates, is the possession of tendencies toward polyisomerism and subsequent anisomerism, which produced some interesting

convergences but never overcame the profound differences in ground-plan between two widely separated phyla.

We must also dismiss the tunicates as being in no real sense direct intermediates between the vertebrates and their unknown invertebrate ancestors for the reason that they may rather be derived from the ancestors of *Amphioxus* than the reverse. The "vertebrate" characters of tunicates seem to be all secondary to a very early stage of attachment after still earlier stages of free-swimming and approximate bilaterality. The floating salps and their allies retain the deep-seated anisomerism or inequalisation of their twisted sessile ancestors. Thus as a class the tunicates demonstrate what startling transformations in organic designs take place by the interweaving phases of earliest larval polyisomerism, adult anisomerism of sessile forms, secondary antero-posterior polyisomerism of floating salps and tertiary radiate polyisomerism of *Octacnemus* (cf. Delage and Hérourard, pl. 35).

The balanoglossids have a distinctly worm-like habitus, and it is probable that some of their characteristics which have been deemed of great importance in determining their remote affinities may be habitus characters of no great geologic age. However, they share with *Amphioxus*, in addition to the technical characters of all chordates, a few peculiar features, such as the presence of tongue bars and crossbars in the branchial supports, the occurrence of an early larval stage with three primary coelomic cavities, and other points noted by Delage and Hérourard (1898, p. 335). Nevertheless, the habitus of adult balanoglossids is highly specialised for a worm-like life of digging in the mud, and we look in vain in their structural pattern for even a remote suggestion of the already highly organised ostracoderms, which are at once the oldest and most primitive of the known fossil chordates.

The presence of the famous *Tornaria* larva in those balanoglossids which have an indirect mode of development (Delage and Hérourard, p. 329) has long been noted as a point of resemblance to certain echinoderms, but inasmuch as these ciliated larvae in both phyla are extremely simple organisms they are not above the suspicion of being produced independently, from the gastrula stage common to many phyla, as a convenient means of dispersal in the search for favourable habitats.

After excluding all the foregoing invertebrate groups from the line of ancestry to the vertebrates, together with the Mollusca, the Moluscoidea, the nematodes, cestodes, rotifers and other unpromising candidates, we have left chiefly the echinoderms. Here it would be difficult to find a wider contrast in organic designs than that between the tree-like, quinquerradiate sea lilies and the stream-lined, actively moving vertebrates. Of course the classic viewpoint is that, on account of the presence of free ciliated larvae in modern echinoderms, the radial arrangement of the adults "imperfectly conceals a more obscure and primitive bilateral symmetry" (Parker and Haswell, 1897, 1, 377), and that the common ancestors of echinoderms and chordates would be essentially like the "*Dipleurula*" larva of echinoderms and the "*Tornaria*" larva of balanoglossids. However, these animated larval seed capsules have yet to demonstrate their right to represent some far-off hypothetical adult ancestor, and the idea that they do so dates from the days when

it was thought safe to accept the merest suggestions of recapitulation without any unpleasant suspicions. The wholesome critical work of de Beer (1930), Morgan (1932) and others, while sweeping away the fogs of speculation, has at the same time shown how embryology may still be of the greatest importance to the student of evolution, since new forms often arise by prolonging larval characters into the sexually mature stages and so gradually retard the appearance of formerly adult characters.

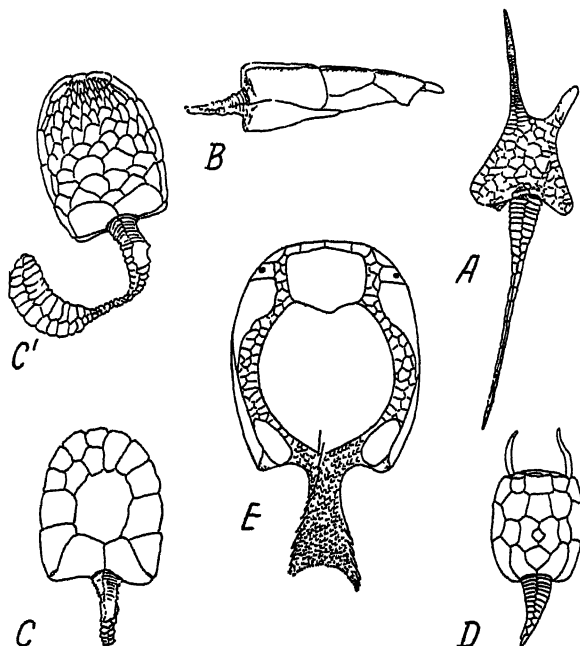


Fig. 2. Anisomerism in certain Palaeozoic echinoderms, involving changes of symmetry and approach to ostracoderm patterns. A, *Dendrocystites scoticus* Bather; B, *Lagynocystitis pyramidalis* Barrande; C, *Mitrocystella Barrandei* Jaekel, lower side; C', *Mitrocystella Barrandei* Jaekel, upper side; D, *Placocystites Forbesi* de Kon. Figs. A-C' from Abel, after O. Jaekel; Fig. D from Abel, after Bather. E, *Drepanaspis* (ostracoderm), after Traquair and Woodward.

While this principle of paedogenesis can be shown to be potent in the origin of certain vertebrates, the idea is not necessary in a comparison of the adult patterns of a typical echinoderm and a typical vertebrate. The former is a fixed stalked form with dorso-ventral asymmetry, and a tendency toward quinquerradiate symmetry. The body is bag-like, and five folds around the mouth are prolonged into "arms". There is little or no brain, but an elaborate system for conducting the food particles along the grooves of the arms to the mouth. The early vertebrate, on the contrary, is a mobile stream-lined form with dorso-ventral and cranio-caudal asymmetry, bilateral symmetry, an extremely active locomotor apparatus, a mouth not homologous with that of echinoderms and a relatively enormous brain.

At first sight it would seem most fantastic to suggest that the sea lily could ever be transformed into the fish, and no doubt the typical crinoids are not in question.

But while such a transformation is as yet far from being demonstrated, it must be admitted that one division of the Palaeozoic echinoderms, namely, certain families included under the subclass Carpoidea by Abel (1920, p. 278), was performing some remarkable experiments in the modification of a quinquerradiate symmetry into a new dorso-ventral asymmetry and a partial bilateral symmetry, and that some of them (Fig. 2) approach in general patterns to the "dorsal" and "ventral" shields of *Drepanaspis* among the heterostracous ostracoderms (Gregory, 1935 a).

The "long arm of coincidence" has of course been able to bring about equally striking but surely fortuitous resemblances between objects that are assuredly farther apart in a phylogenetic sense than are the echinoderms and the chordates (cf. Dean, 1908). Nevertheless, certain ancient echinoderms, which were essentially fixed and quinquerradiate in the adult stage, have been able to give rise to free-moving forms with an approach toward bilateral symmetry and a rather startling suggestion of relationship to the most primitive known chordates. This fact ought at least to free our minds from the tradition that the invertebrate ancestors of the vertebrates must necessarily have been bilaterally symmetrical and metameric forms moving in a cranio-caudal direction like worms and primitive arthropods.

IV. AMPHIOXUS

The traditional right of *Amphioxus* to be regarded as a protochordate in the literal sense was founded largely upon the surpassing simplicity of this organism, which appeared to be an almost ideal archetypal vertebrate, although everyone recognised that certain of its characters, such as the prolongation of the notochord to the tip of the snout and the resultant asymmetry of the larval mouth and gill openings, might well be specialisations. Patten (1912, figs. 176-8) appeared at first sight to hint that *Amphioxus* might be a greatly simplified derivative of such a complex form as *Bothriolepis*, but a closer reading of his text and other diagrams (pp. 397, 471) shows that he regarded *Amphioxus* not as a primitive vertebrate but as an "Acraniate", which was not as closely related to the vertebrates as were the primitive arachnids. If this were true, however, a good part of the "vertebrate" characters of *Amphioxus* would be due to parallelism, a thought which should have embarrassed one who would not admit convergence between the vertebrates and the arachnids.

Delage and Hérouard (1898, p. 337) concede to *Amphioxus* many important marks of affinity with the vertebrates but end by placing it "en tête de l'embranchement des Procordés qui fait le passage des Invertébrés aux Vertébrés". However, this phrase reflects the almost eighteenth-century Cuvierian viewpoint of these authors, who were extremely sceptical toward attempts at reconstructing lost common ancestors between vertebrates and invertebrates, and therefore regarded the position assigned to *Amphioxus* in their classification as expressing the net result of their elaborate morphological analysis, which had exposed significant agreement of *Amphioxus* on the one hand with the balanoglossids and tunicates and on the

other with the larval vertebrates, especially the cyclostomes. At the present time, however, the great expansion of our knowledge of the morphology of the ostracoderms demonstrates that the earliest true vertebrates were already possessed of a strongly developed exoskeleton and a highly complex brain.

Specialised vertebrates of many groups are frequently characterised by a high degree of secondary polyisomerism in certain organs, coincident with extreme anisomerism, either positive or negative, in others. In *Amphioxus* we may safely infer that there has been extreme secondary polyisomerism in the branchial arches and in their subdivision by tongue bars and connecting bars; this tendency toward multiplication is further seen in other parts of the organism, *e.g.* in the myomeres, the giant nerve cells of the spinal cord, and in all the components of the notochord. Here is a good example of the fact that anisomerism or change in dimensions inevitably results from polyisomerism, since in this case the multiplication of the elements of the notochord has brought about its great increase in length and its doubtless secondary extension to the tip of the snout. Negative anisomerism is illustrated in the reduction of the brain to almost vanishing dimensions and to an extreme simplicity, which bears rather the suggestion of degeneration from a typical vertebrate brain than the stamp of the true primitiveness of a nascent chordate. Further losses are suggested in the complete absence of internal ears (otic capsules) and in the absence of either paired or pineal eyes; but scattered retinal elements may perhaps be represented by the few widely dispersed light cells in the brain and spinal cord.

The complete absence of an exoskeleton in *Amphioxus* may no longer be considered a primitive character, since all the experience of palaeichthyologists supports the conclusion that the later vertebrates have been derived from forms with a well-developed exoskeleton. On the other hand, the hypothesis that *Amphioxus* had been derived from some of the ostracoderms by degeneration of the exoskeleton, severe reduction of the brain, hypertrophy of the notochord and multiplication of the branchial parts would all be consistent with the retention of such extremely primitive-looking chordate characters as the segmental nephridial organs and gonads, the separateness of the dorsal and ventral branches of the spinal nerves and so forth.

Thus we come to the disturbing thought that while the mode of development of the mesoderm and coelome of *Amphioxus* has always been assumed to be ideally primitive, it is highly probable that the embryology of the cyclostomes is a safer guide to the embryology of the oldest true chordates, which are the ostracoderms, and that the embryology of *Amphioxus* may be as much secondarily simplified as is its adult morphology.

However, after discounting the value of *Amphioxus* as a true intermediate between vertebrates and invertebrates, and notwithstanding my strong suspicion that it has been derived by degeneration and specialisation from some of the known Palaeozoic chordates, I would not underestimate its historic value in providing a simplified picture of chordate development and adult anatomy. Moreover, the many classic studies on the embryology of *Amphioxus* show how by the continuous

co-operation of the simple principles of repetition and emphasis a zygote (itself the product of the same processes) gives rise eventually to an adult anatomy of considerable complexity.

V. ORIGIN AND DEPLOYMENT OF THE AGNATHOUS CHORDATES

As long as zoologists pictured the ancestors of the vertebrates as eel-like, naked and almost brainless forms essentially like *Amphioxus*, they left too largely to the palaeontologists the priceless material of the ostracoderms, which if not pre-vertebrates are at least pre-fishes. However, Cope, Goodrich and possibly others did not fail altogether to grasp the importance of the ostracoderms, but it was not until the superb material and fundamental studies by Kiaer and Stensiö were available that the demonstration of the central position of the ostracoderms in the major problems of the origin and deployment of the earlier vertebrates became apparent.

The outstanding feature of typical ostracoderms as compared with invertebrates is that the head is of essentially vertebrate type with three pairs of capsules (olfactory, optic, otic) arranged in a cranio-caudal series, the last pair being on either side of the front end of the notochord. All three pairs, with the cranial nerves, lie above a series of paired branchial pouches; there is a median slit-like mouth in series with the circular paired gill openings. Jaws if present were small dermal plates not connected with the visceral arch series (Kiaer). The head (including the branchial organs) was usually of large size and protected by a "shield" of several layers including an outer layer of dentine, the shield either continuous or consisting of individual plates or small "placoid" scales. The surface of the shield bears rows of supposed lateral-line organs arranged in characteristic patterns in different families.

The locomotor organs consist of folded myomeres, separated by myosepta, running along the flanks and covered with plates or scales of the same nature as the head shield. The tail is hypocercal, that is, the notochord is turned downward and the membranous tail lies above it. Median fins if present originate from projections of the large scales on the body.

It has been noted above that such a generalised picture of an ostracoderm differs so widely from that of any arachnid or other arthropod that there is no justification for assuming a relationship merely because both groups exhibit repetition of locomotor segments with subsequent coalescence of certain neuromeres, for the entire anatomical pattern of the head is extremely different in the two phyla.

When fully developed the exoskeleton of ostracoderms consists of five layers: (a) a basal laminated layer; (b) a thick layer of large chambers or cancellae lying between trabeculae; (c) an irregular vascular or reticular layer traversed by blood vessels; (d) a layer of dentine denticles or tubercles with pulp cavities; (e) a thin outermost layer of epidermis. The class is divided into four orders: Heterostraci (Pteraspidomorphi), head shields without bone cells; Osteostraci (Cephalaspidomorphi), head shields with bone cells; Thelodonti, with the shield broken up into

many individual dentine tubercles; Anaspida, with small delicate head shields of many pieces, bodies elongate, sinuous.

The oldest known fragments of head shields of ostracoderms occur in the Harding Sandstone (Middle Ordovician) near Canon City, Colorado, and were named *Astraspis desiderata* by Walcott. According to Eastman (1917, p. 238) the large median dorsal and median ventral plates of the shield are "of compound nature, being made up of a large number of small polygonal tesserae rising into a conspicuous central prominence which is surrounded by minute stellate tubercles". Here evidently we have to do with polyisomeres conjoined into large compound poly-anisomeres.

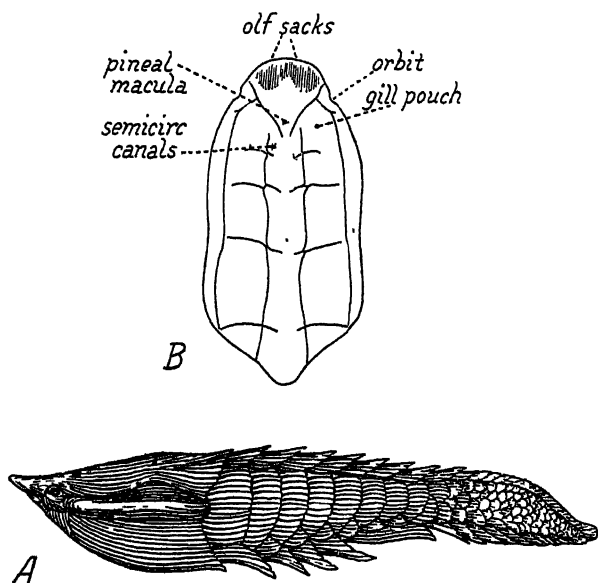


Fig. 3. Anisomerism and polyisomerism in the most primitive known chordates. A. *Anglaspis heintzi* Kiaer, a poraspid ostracoderm from the Downtonian of Spitzbergen. After Kiaer. This form in its general plan is an extremely primitive vertebrate. The surface ornamentation of the head shield and scales, on the other hand, illustrates the wide-spread phenomenon of secondary polyisomerism, whereby comparatively new features found in one part will also be found in others in series with it. B. Head shield of a primitive heterostracous ostracoderm, *Poraspis polaris*. After Kiaer. Dorsal view, showing positions of the impressions on the inside of the dorsal shield and the course of the sensory canals (heavy black lines). The ovals represent the gill pouches.

According to the mature and broadly based conclusions of Kiaer (1932, pp. 10-12) the head shield of the most primitive Heterostraci was not subdivided into numerous small scale-like plates but consisted chiefly of four plates: a large undivided, vaulted, median dorsal shield, a pair of laterally placed, elongated, marginal "branchial" plates, an undivided, median ventral shield. The flattening of the ventral shield implies temporary resting on the bottom. Certain forms also had a small suborbital plate under each of the paired eyes, while the mouth is known in one or two forms to have been bordered by small plates, probably embedded in a movable oral margin (Kiaer, 1928).

Fig. 3 B, after Kiaer, shows the relations of the rows of lateral-line organs to the central nervous system and to the branchial pouches and openings in a relatively primitive form, *Poraspis*.

In *Dictyaspis complicata*, which is believed by Kiaer (1932, p. 17) to be both later and more specialised than *Poraspis*, secondary polyisomerism has affected the lateral-line system, which is broken up into an irregular network. In *Anglaspis* (Fig. 3 A) the longitudinal "ribs" on the surface of the undivided dorsal and ventral shield show a tendency to divide the shield into various areas (Kiaer, 1932*b*, pl. VII), while in *Tolypaspis* (pl. X) the dorsal shield tends to break up into very many small shield-shaped areas. A continuation of the process of secondary polyisomerism would yield the innumerable small placoid tubercles of *Thelodus*, or the denticles of *Lanarkia* and of *Coelolepis*. Hence these forms, although presenting an appearance of extreme simplicity and lack of regional anisomerism, were not regarded by Kiaer (1932*b*) as truly primitive.

The coelolepids (of the order Thelodonti) have a short bag-like body with very small eyes, a small mouth and no gill openings. The prey may have been drawn in by ciliary action or sucked in by the muscular gill pouches.

From such relatively sluggish forms as this the tunicates might have been derived after extensive degeneration and specialisation (anisomerism). On the other hand, a heterostracan, *Anglaspis heintzi* Kiaer (Fig. 3 A), is a fusiform and free-swimming type in which the head shield is stream-lined into the stout body.

The older cyathaspids have very short to short rostra, but among the pteraspids the rostrum may become long (as in *Podalaspis*). There is also marked range of variation in the relative width of the dorsal head shield as compared with its length, from the narrow, elongate *Eoarchegonaspis wardelli angusta* to the broad rounded disc of *Drepanaspis*. Thus anisomerism co-operated with polyisomerism in giving diversity to the heterostracous pattern.

According to Kiaer, a pair of depressions on either side of the midline on the ventral surface of the rostral shield represent paired olfactory sacs (Fig. 3 B). Partly on this account Kiaer has referred the order of Heterostraci to the major series Diplorhini, involving the elasmobranchs and higher vertebrates, in contrast with the Monorhini or forms with but a single olfactory sac, including the Osteostraci and the modern lampreys. Hence it is conceivable that Traquair's judgment that *Thelodus* and *Lanarkia* were also related to the stem of the sharks may be confirmed, but Kiaer has shown that in the heterostracan *Pteraspis* the dermal jaw plates were not supported by endoskeletal pieces in series with the branchial arches, hence that the functional jaws of *Pteraspis* were not of the gnathosome or gill-arch type but of the agnathous or cyclostome type. Moreover, by placing such an extremely high value on the doubleness or singleness of the olfactory sac and certain other features both Kiaer and Stensiö have had to separate very widely the modern myxinoids (hagfishes) from the modern petromyzonts (lampreys), the former being included with the Diplorhini, the latter with the Monorhini. But space is lacking here to set forth the evidence that in spite of the differences cited above

and others the myxinoids and the petromyzonts are after all divisions of a fairly unified group, the cyclostomes.

In the second great order of ostracoderms, namely, the Osteostraci or Cephalaspidomorphi, the head shield is expanded in front into a semicircular disk, domed in the middle and surmounted by a pair of spectacle-like eyes set near the midline; in front of the paired eyes is a small circular depression containing a median slit, the unpaired olfactory opening. The head shield is distinguished from that of the Heterostraci by the presence of bone cells, especially in the laminated and trabecular layers. The brain, cranial nerves, blood vessels and the semicircular canals are represented in many of Stensiö's specimens both by natural casts of their chambers and tubes and by serial sections that reveal a host of illuminating details.

From Stensiö's profound studies on this group (1927) emerge the following great generalisations:

(1) The cephalaspid cranial nerves, blood vessels, semicircular canals and chief divisions of the brain are identifiable by comparison with the corresponding parts in modern cyclostomes, especially the larval petromyzonts.

(2) The front part of the cephalaspid head shield represents a great expansion of the posthypophysial fold of the roof of the mouth of the larval lamprey and clear remnants of the dorsal shield are present in the roof of the suctorial chamber of the adult petromyzonts.

(3) The modern petromyzont is a secondarily naked, elongate and eel-like form which has acquired a suctorial and rasp-like mouth and predaceous habits.

(4) The class Ostracodermi, together with the modern class Cyclostomi, is united under the superclass Agnatha, which is called "jawless" in allusion to the fact that their jaws, if present, were not connected with the visceral (branchial) arches, in contrast with the superclass Gnathostomi, including all the remaining vertebrates, with primary jaws of gill-arch derivation (Fig. 4).

At first sight the arrangement of the cranial nerves and blood vessels seen in Stensiö's cephalaspids seems bewildering and astounding in its complexity. But on closer inspection we realise that in the case, for example, of the so-called "electric" nerves, although many of these branches were even at that remote time already secondary polyisomeres (connected in part with the great expansion of the posthypophysial fold), they had not yet been affected by the prolonged anisomerism which in the cranial nerves of a modern lamprey has led to the entire suppression of these "electric" nerves and has brought about a secondary simplification.

The Osteostraci exhibit a considerable deployment in the shape of the head shield by simple anisomerism (Fig. 5); first in the breadth-length and breadth-height relations, second, in the length of the posterior cornua of the shield, third, in the extent of the so-called electric field, and the like. Apparently secondary polyisomerism is indicated in the verrucose ornamentation of the shield in *Thyestes* and related genera (Kiaer, 1930, p. 5), as well as in the reticulate character of the shield in *Ateleaspis*. *Tremataspis*, as described by Patten (1903), is distinguished by the apparently secondary breaking up of the "electric field" areas on the dorsal shield, as well as by the suppression of the lateral cornua and their associated

pectoral flaps and embayments. The throat in this genus is protected by a system of movable plates, but there is no convincing evidence that the mouth was anything but the terminal transverse slit in series with the small gill openings.

Patten (1903) believed that at least certain of the supposed gill openings in *Tremataspis* were the sockets of paired appendages which had an exoskeleton of the same structure as the head shield, but this idea is quite inconsistent with definite evidence from other ostracoderms that these small openings were connected with

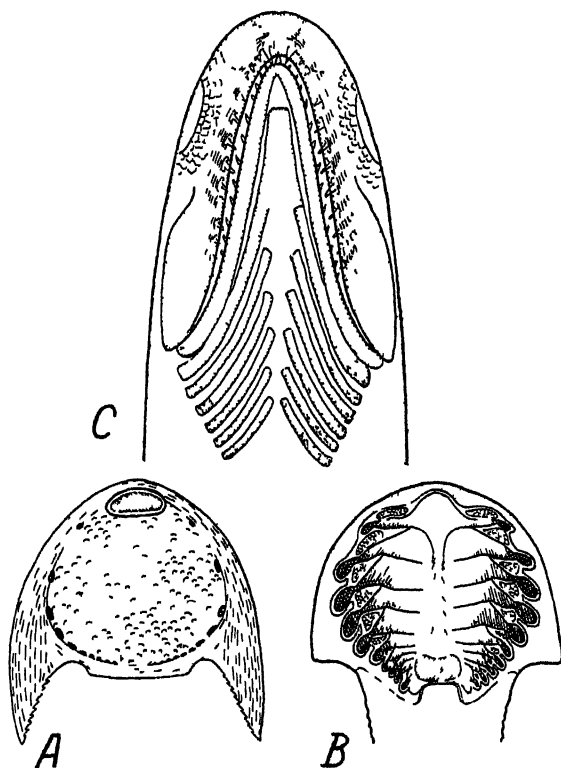


Fig. 4. Polyisomerism in primitive Agnatha and Gnathostomata. A. Under side of head of ostracoderm, showing supposed position of mouth and gill openings. Restoration after Stensio. B. Under side of head shield of ostracoderm, showing roof of oralo-branchial cavity with dorsal part of branchial skeleton. None of the gill arches is enlarged to serve as jaws. Restoration after Stensio. C. Under side of head of palaeozoic shark (*Cladoseleache*), showing enlarged jaws in series with gill arches. After Dean.

the branchial sacs and were in series with the mouth. Equally mistaken was Patten's identification (1912) of certain paired appendages of primitive arachnids with the "balancers" or epibranchial organs of dipnoans and urodeles. Thus the simple principles of repetition and emphasis often produce fortuitous and misleading resemblances in details between organic designs of fundamentally different origins.

The Anaspida, fourth and last order of ostracoderms (Kiaer, 1924), are not strictly shieldless, as their name implies, but have the dorsal head shield small and

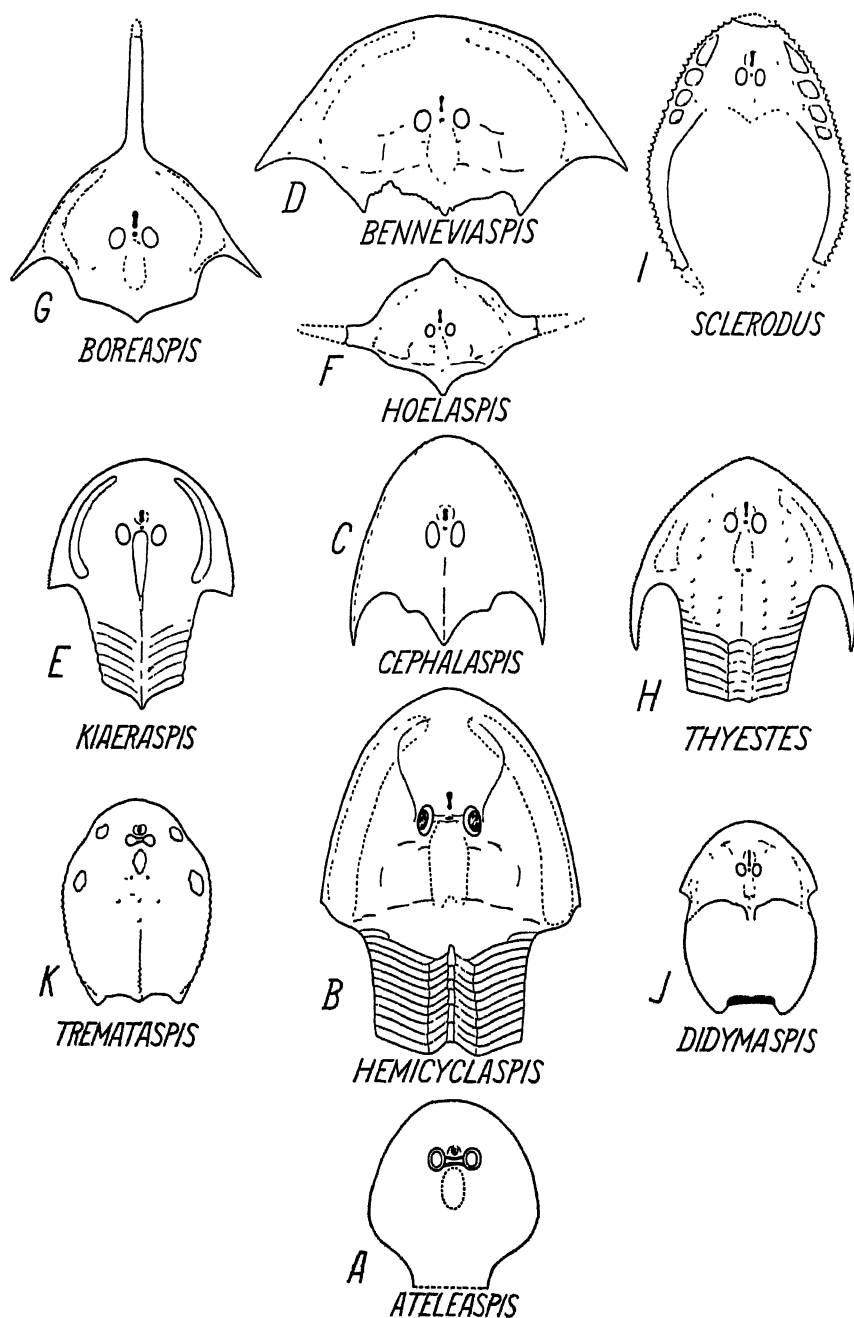


Fig. 5. Anisomerism of the head shield among the cephalaspids. Scales various. A, after Traquair. B, D, E, F, G, H, I, J, after Stensiö. G is probably less specialised than F. C, after Powrie and Lankester. K, after Patten.

broken up into small thin plates arranged around the rostral, pineal and orbital openings (Fig. 6). The expanded throat is covered with numerous small oat-shaped scutes; there are vertically high delicate scutes on the flanks of the sinuous body. All these features as seen in the light of Kiaer's researches have been derived by secondary polyisomerism and anisomerism from some primitive cephalaspid. Certain of the Anaspida, namely *Lasanius*, had become specialised to the stage of reduction (negative anisomerism) and more or less complete elimination of the scales on the flank, with emphasis of a row of triangular scutes on the dorsal midline. The tail was turned downward (hypocercal) as in cephalaspids, the web of the tail being located above the notochord; incipient paired fin-folds were supported by spines on their anterior borders.

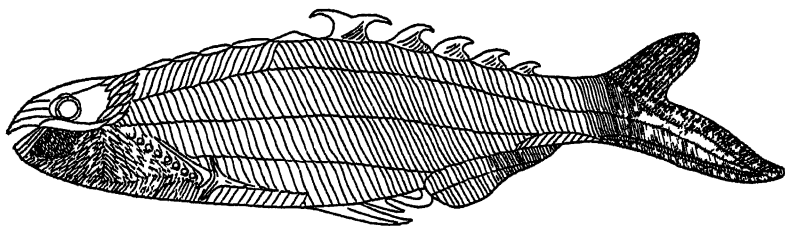


Fig. 6. Interregional anisomerism combined with intraregional secondary polyisomerism. Revised restoration of anaspid ostracoderm, *Birkenia elegans*. After H. C. Stetson.

According to Kiaer some of the Anaspida may have given rise to the modern lampreys, a transformation requiring further secondary polyisomerism of body segments, reduction and elimination of the exoskeleton, further development of the rasping "tongue" (=mandible), which had reached only an incipient stage in *Pharyngolepis*. As intimated above, the myxinoids (hagfishes) have only carried such degenerations and specialisations much farther than the lampreys, so that in spite of their widely different embryology it seems difficult to regard them as independent derivatives on the one hand of the Anaspida (lampreys) and on the other of the Heterostraci (myxinoids).

VI. EMERGENCE OF THE JAW-BEARING VERTEBRATES

The most highly evolved of the Agnatha, which are the cephalaspids, are definitely connected with the stem of the existing lampreys and therefore cannot be close to the widely different jaw-bearing vertebrates. But the same objection does not appear to apply to the heterostracous ostracoderms, which are referred by Kiaer to the Diplorhini, a superclass group of chordates characterised by the presence of paired olfactory sacs and including, besides the Heterostraci, the true jaw-bearing vertebrates. Now, as is well known, the primary jaws of the Gnathostomi are homologous with branchial arches, but such heterostracous ostracoderms as *Poraspis* (Fig. 3 B) and *Cyathaspis* show us branchial pouches in an exceedingly primitive stage, before the elaboration of branchial arches. Hence *Poraspis* and other primitive heterostracous ostracoderms are about the nearest stage that we have to a structural forerunner of the jaw-bearing vertebrates.

(1) *Placoderms*

One of the greatest and most important puzzles in the deciphering of the evolutionary rise and deployment of the lower vertebrate classes is afforded by a strange-looking fossil fish from the Old Red Sandstone known as *Pterichthys*. The parts of this anomalous creature look as if they had been assembled from such incongruous sources as crustaceans and tortoises. The tortoise, we might imagine, had contributed the domed carapace and flat plastron, while some crustacean might have furnished the pair of long and jointed swimming appendages which are covered with a stout exoskeleton but have no endoskeleton.

Bothriolepis, the American cousin of *Pterichthys*, helped on the illusion by revealing a pair of globular eyes projecting from the top of the carapace almost after the manner of the paired eyes of *Limulus*. To such a degree and in so many ways does *Bothriolepis* convey a suggestion of arthropod relationship that it was naturally chosen by Prof. Patten as one of the main pillars for his bridge between arachnids and vertebrates. But, as we have seen above (p. 326), the other main pillar formed by the ostracoderms has been undermined by Stensiö's demonstration that the cranial nerves and brain of the cephalaspids are so fundamentally unlike those of any arthropod as to afford decisive evidence against the derivation of the former from the latter. As to the antiarchs, Stensiö's intensive studies of their anatomy (1931) clearly imply that the supposed arthropod characteristics, especially of the appendages, are not found in the more fundamental plan but in superficial details that are not beyond the scope of convergent evolution.

More difficult questions are the following: (a) How are the Antiarchi related to the Arthrodira? (b) How are they related to the ostracoderms? We shall presently see that Stensiö has been able to prove that the Arthrodira belong with the true jaw-bearing vertebrates (Gnathostomi), and to adduce strong reasons for regarding the peculiar jaw plates of Antiarchi as completely homologous with those of the Arthrodira. In brief, Stensiö definitely refers the Antiarchi also to the Gnathostomi and in so doing implies a very wide separation from the Agnatha.

If *Phyllolepis* of the Upper Devonian could still be referred to the Heterostraci it would be possible to regard it as a kind of structural link between the Heterostraci and the Arthrodira, since it shares with the former a considerable superficial resemblance; but Stensiö (1934) has demonstrated that *Phyllolepis* represents an otherwise unknown order of Placodermi allied with the acanthaspids and true arthrodires. In other words, so far as the fossil record is known, there is a wide gap between the ostracoderms and the Arthrodira. Nevertheless, although *Phyllolepis* itself is already a specialised side branch of the Arthrodira, it still suggests that the entire Antiarchi-Arthrodira series, of which the oldest known stages had well-developed pectoral spines, have been derived eventually from such primitive Heterostraci as *Poraspis*, possibly by the development of spiny processes in the front corners of the median ventral plate. And in spite of the difficulty of equating individual plates in acanthaspids and Heterostraci, there is a strong suggestion of remote relationship even in the arrangement of the lateral-line canals.

To one who has watched the chequered career of the Arthrodira during the past thirty-five years and has seen them variously allotted to the Dipnoi, to the ganoids and to a separate class, Arthrognathi, characterised by the lack of true jaws of normal vertebrate type, it has occasioned no little rejoicing that Stensiö (1934) has been able to confirm Jaekel's thesis (1907, 1919) that the Arthrodira are true jaw-bearing fishes. For certain specimens of Jaekel's, *Pholidosteus* and *Leiosteus*, described by Stensiö, bear the peculiar antero- and postero-supernathals and infragnathals of the Arthrodira firmly attached respectively to a palatoquadrate arch in the upper jaw and to a true Meckel's cartilage in the lower, essentially as in the sharks and ganoids. Thus at one stroke speculation is replaced by irrefutable fact. And the series of diagrams (Fig. 7) by Heintz (1931) illustrating the ultimate origin of the typical gigantic Arthrodira from small forms of the order Acanthaspida affords a striking example of the principle that selective emphasis (anisomerism) finally accumulates into a transformation.

From all the labours of many workers on Arthrodira we may visualise a grand adaptive radiation of a very early side branch of the jaw-bearing vertebrates, starting perhaps with small forms not unlike the ostracoderm *Poraspis* without true jaws, increasing in size and becoming predatory as the superficial jaw plates gained the backing of one of the enlarged gill arches; finally attaining gigantic size, losing the teeth and developing cutting shears on the edges of the jaws or flattening the tips of the jaws into either prehensile (*Titanichthys*) or crushing plates (*Mylostoma*).

(2) *Elasmobranchs*

All recent work in palaeichthyology supports the conclusion that the most primitive known true fish is not the long-bodied, spineless shark *Cladoselache* of the Upper Devonian but some of the older, short-bodied, broad-spined acanthodians such as *Diplacanthus*. To make a very long story unduly short, we may accept the evidence afforded by the known ostracoderms, placoderms and earlier elasmobranchs in preference to the "hypothetical primitive vertebrate with continuous fin-folds", which is an anachronism that still persists in some text-books.

Among later sharks we observe in some lines a tendency to reduce and eliminate the strong spines on the front borders of the fins, which are found in the oldest known sharks. Even among the Devonian cladodont sharks one form, *Ctenacanthus clarkii* (Dean, 1909, p. 251) retains a large spine on the dorsal fin but has already lost the spines on the remaining fins, while the more advanced *Cladoselache* represents a stage soon after the loss of all spines.

This genus, long famous in the literature of the origin of paired fins, is now seen to represent a stage in which there was a marked secondary polyisomerism of the basal rods of all the fins, accompanied in the pectoral region by anisomerism, including crowding and fusion of some of the rods into a scapula and three-piece base, structurally ancestral to those of modern sharks. Thus *Cladoselache*, instead of being more primitive in its fins than the ancestral acanthodians, stands structurally between the acanthodian stem and later sharks.

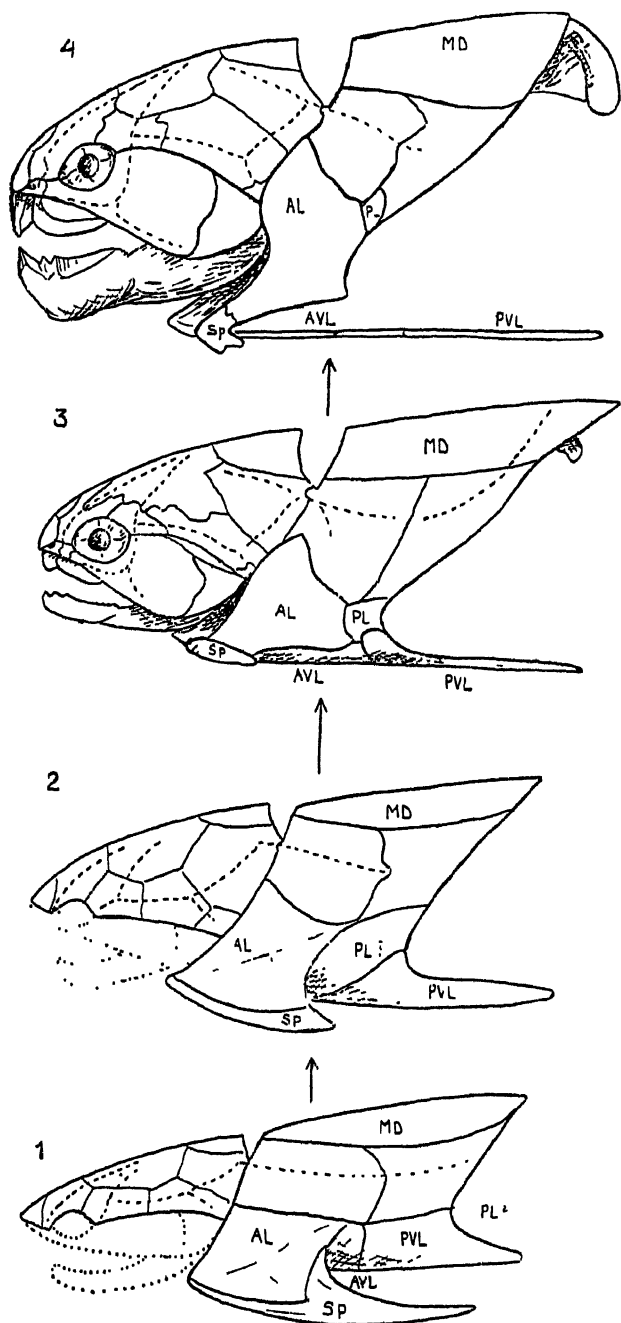


Fig. 7. Progressive anisomerism in the evolution of the Arthrodira. After Heintz. 1, *Acanthaspis*; 2, *Phlyctaenaspis*; 3, *Coccosteus*; 4, *Dinichthys*.

A comparative study of the ostracoderms, placoderms and earlier elasmobranchs suggests that both median and paired fins were developed *in situ*, with the exception that the pectorals and pelvics were once connected by intermediate spines and nodes somewhat as in certain acanthodians (e.g. *Climatius*). The muscles of the fins, both median and paired, are known from the embryology of recent sharks to be derived from localised buds of the myomeres, and the fossil *Cladoselache*, in which the muscle tissue is often preserved, shows the myomeres extending on the base of the fin exactly as in modern sharks.

In the true sharks the endoskeleton of the fins was emphasised, while the exoskeletal spines usually became reduced or eliminated. In the later acanthodians the reverse was true. But as Dean (1907) has shown, in the earlier acanthodians there were short basal rods in the fins, presumably developed *in situ* as a response to the muscles (derived from the myomeres) which moved the fins. In the later and more specialised acanthodians, however, the basal rods were finally eliminated (except in the paired fins), and whatever muscles remained were fastened to the bases of the long, delicate spines.

The spines of these early sharks illustrate a principle of far-reaching scope, whereby any fine details of structure or sculpture which may be observed on one of the parts of any given species is usually to be seen on all other homologous parts of the same species. This likeness of appearance in adjacent parts often brings about a degree of similarity which in many instances has been mistaken for a sign of an undifferentiated primitive condition. I have called this method by which organic designs change *secondary polyisomerism*. Even primitive polyisomerism often exhibit secondary polyisomerism in details (see p. 339 above).

The pleuracanth, which were extremely specialised fresh-water sharks of late Palaeozoic time, well illustrate the confusing effect of secondary polyisomerism carried to a high degree. We may be sure, however, that the high number of vertebral segments and the still higher number of the rods supporting the greatly elongate dorsal fin in this fish are all secondary.

The pectoral fins of the pleuracanth are also of interest because they exhibit the cumulative results of the following processes: (1) an earlier period of strong regional anisomerism, manifested in the crowding and concentration of the base of the paddle into a movable wrist-like base; (2) a process of secondary polyisomerism, evident in the extreme reduplication of the central axis of the fin and of the feather-like peripheral rays. An even more rapid centrifugal growth of the pectoral fin has doubtless been attained independently in the Antiarchi also and in the crossopterygian-dipnoan stock. The pelvic fins of *Pleuracanthus* are somewhat more like those of other sharks and have undergone less rapid transformation, although already affected to a considerable extent by secondary polyisomerism.

In the region of the "visceral arches" the elasmobranchs, including the acanthodians, afford a convincing demonstration of the origin of primary upper and lower jaws from earlier gill arches. This profound transformation of form and functions has long been well known to embryologists since the time of Reichert. The detailed evidence for the view that the upper and lower oral arches have arisen

from a series of polyisomeres (the gill arches) by anisomeric emphasis of certain parts may not be reviewed at this point except to note that the oral arch of elasmobranchs and even of higher fishes strongly resembles the arches behind it in its relation to surrounding tissues (especially in the embryo), as well as in the arrangement of its muscles, which are represented by the deep flexors of the branchial arches.

By secondary polyisomerism of the denticles of the skin around the mouth, teeth were, so to speak, invented; by subsequent intradental polyisomerism the many-cusped teeth of some later sharks were produced.

Stensiö (1927, 1934) has adduced much evidence for his conclusion that the "cartilaginous" skeleton of recent sharks is retrogressive, due to delay in the ossification of the endoskeleton and consequent retention of larval characters. Such retardations come under the heading of negative anisomerism, and it is evident from this one example, out of very many that could be given, that anisomerism (unequal emphasis) always arises from accelerations, retardations, prolongations or abbreviations of growth rates in ontogeny and that such changes finally spread to entire classes in phylogeny.

Thus notwithstanding the many primitive characters retained by sharks it is no longer believed that any known sharks are directly ancestral to the higher vertebrates. Even the oldest known acanthodians are probably not the direct ancestors of the ganoids, crossopterygians, dipnoans and amphibians of later ages. But they are probably much nearer to such ancestors than is the "hypothetical primitive vertebrate" of the text-book.

The first gnathostomous or true jaw-bearing vertebrates probably antedated by a considerable geologic period the earliest recorded fossils of "sharks", ganoids, crossopterygians and dipnoans, for by the time of the Old Red Sandstone representatives of true sharks, primitive ganoids, typical crossopterygians and dipnoans had already become well differentiated and were living side by side with the later members of the ostracoderms and placoderms. Nevertheless we may recall from what precedes that the forerunners of the typical jaw-bearing vertebrates must have been related to the ancestors of the aberrant class of placoderms, and further that they must have been derived ultimately from near the stem of the heterostracous ostracoderms; from this point we can look forward over a few of the principal steps that took place in the origin and deployment of the more typical vertebrates, which are the modern teleost fishes, the amphibians, reptiles, birds, mammals and man.

The later sharks differ from the earliest sharks chiefly in the emphasis and retention of the cartilaginous skeleton, with the addition of calcium salts. In the early ganoids, on the other hand, bone cells usually remained conspicuous both in the endoskeleton and in many parts of the dermal skeleton, such as the dermal rays of the fins. In the sharks the outer webs of the fins are formed from very fine keratin rays; in the ganoids the fin rays arise from the fusion of small scales with a bony base and a coating of ganoin; this is gradually replaced in the teleosts by horny material.

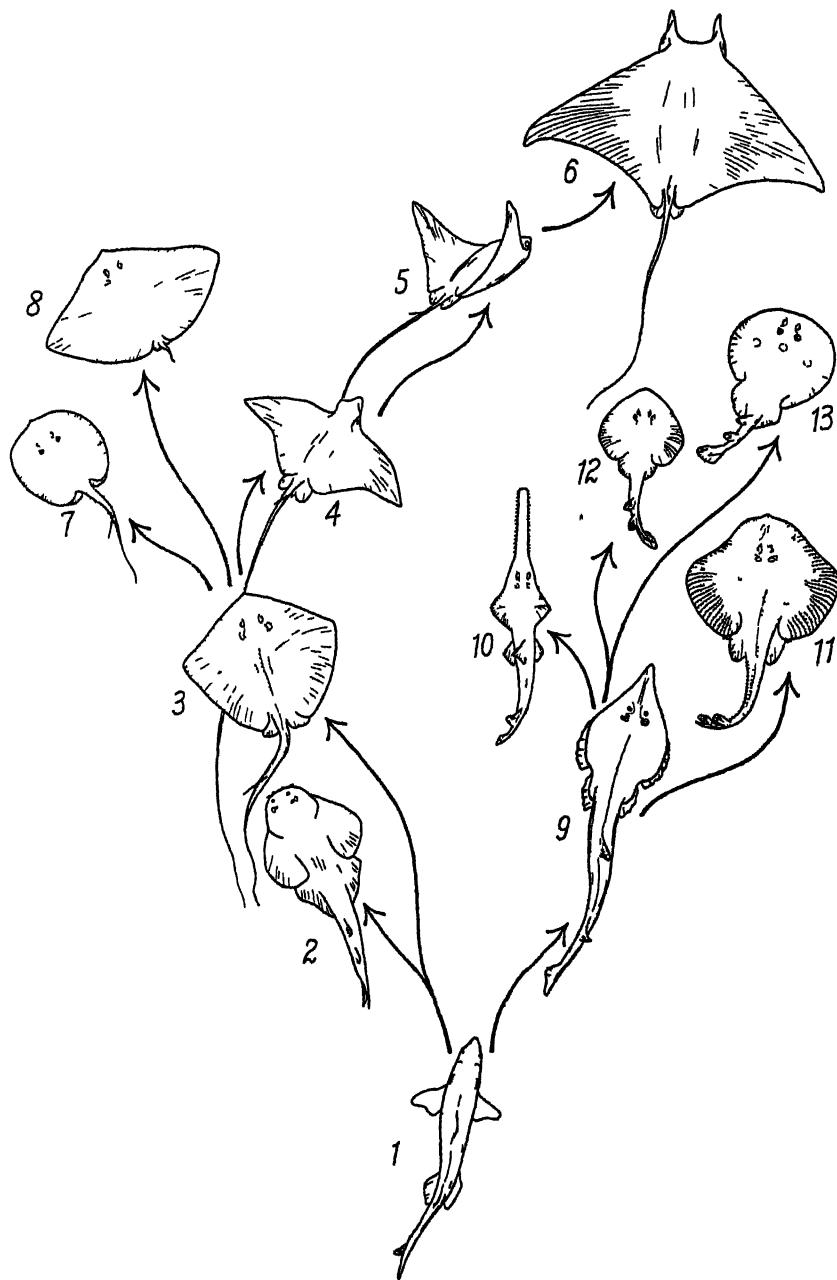


Fig. 8. Divergent anisomerism in the skates and rays. 1, spined shark (*Squalus acanthias*); 2, monkfish (*Squatina squatina*); 3, sting ray (*Dasybatus hastatus*); 4, spotted eagle ray (*Aetobatus narinari*); 5, cow-nosed ray (*Rhinoptera quadriloba*); 6, small devil-fish (*Mobula olfersi*); 7, river ray (*Potamotrygon circularis*); 8, butterfly ray (*Pteroplatea mucrura*); 9, Japanese guitar-fish (*Rhinobatus schlegelii*); 10, sawfish (young) (*Pristis pectinatus*); 11, common skate (*Raja erinacea*); 12, Chinese fan-fish (*Discobatus sinensis*); 13, European electric ray (torpedo) (*Narcacion nobilhanus*).

The sharks played many anisomerous variations on the primitive body form, lengthening it at one extreme into the almost eel-like *Chlamydoselache* but emphasising the transverse diameter of the pectoral fins to an extreme degree in the typical skates and rays (Fig. 8).

VII. DEPLOYMENT OF THE HIGHER VERTEBRATES

The ganoids and teleosts display still greater changes in the stream-lined body form (Fig. 9). Even more astounding, however, are the transformations of the locomotor apparatus in the vertebrates after the pectoral and pelvic paddles of

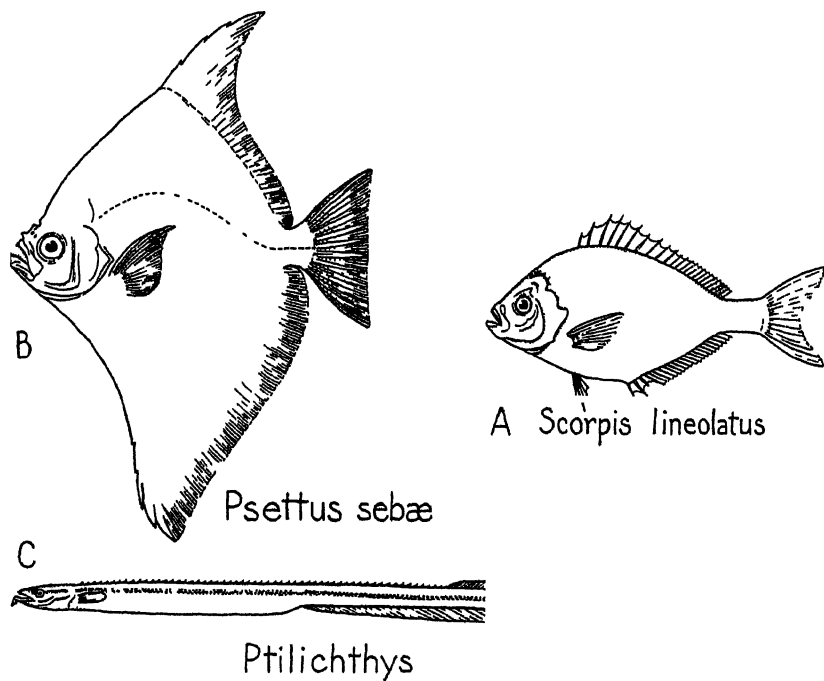


Fig. 9. Divergent anisomerism in percomorph teleosts.

the crossopterygians became changed into the central five-rayed type of hands and feet. Here one need mention only the many adaptations for running, the different ways in which flight through the air was achieved and the numerous independent assumptions of fish-like form in aquatic reptiles and mammals. At every point the evidence plainly indicates that even such an excessively complex organic design as that illustrated in the skeleton of the humming bird has been derived from the far more simple pattern of the skeleton of the earliest chordates by the accumulation and integration of interweaving phases of anisomerism and polyisomerism.

With regard to the skull, the sharks elected, so to speak, to emphasise the internal cranium at the expense of the dermal skeleton, but in the earliest ganoids

(*Cheirolepis*), crossopterygians and dipnoans we find the true or internal brain case well covered by a bony cephalic shield extending over the whole face, cranium and cheeks, opercular region, throat and pectoral girdle. It is by the anisomerous emphasis, fusion, reduction and fenestration of different parts of this bony cephalic shield that the most diversified forms of skull in the higher classes of vertebrates have been derived.

The varied temporal openings and arches of the reptiles, birds and mammals have arisen, in ways now quite definitely understood (Gregory and Adams, 1915; Case, 1924), from the continuous cephalic shield of primitive jaw-bearing vertebrates through the co-operation of two subprinciples of anisomerism: first, the absorptive action of muscle masses on underlying bony plates, which frequently results in a perforation of the central area and a building up by trabeculae of the periphery; second, the emphasis of any one of the temporal openings and bars at the expense of the others.

The protean modifications of the upper and lower jaws (Gregory, 1927, 1929) likewise illustrate the moulding effect of muscle upon bone; the latter reacting, on the one hand, passively, being often pushed out of the way by invading muscle; on the other hand, actively, by building up bony trabeculae at right angles to the lines of stress. Only a single example of these principles may here be cited. The mandible of the oldest crossopterygians forms a complex organic design, consisting of an internal core, derived from the Meckel's cartilage or inner primary jaw, surrounded by a system of bony plates on both the lateral and medial surfaces of the mandible. From the eighteen elements included in the mandible of the crossopterygian only one pair, the opposite dentary bones, survives in the adult mandible of mammals. Anisomerism has favoured this pair and eliminated the others as jaw-bearing elements (Fig. 10); but one pair of these elements that were eliminated from the masticatory functions was retained, and through an enormous change of function has survived in the form of the malleus or hammer bones of the middle ear. Space is completely lacking to set forth the evidence for the reality of this virtual miracle of transformation, but the proof of it was the life work of a great morphologist, E. Gaupp.

The greatest transformations during the origin and deployment of the higher vertebrate classes took place, however, in the methods of reproduction and development, in the ways of maintaining and stabilising the body temperature, and in the patterns of the central nervous system, each a vast but fascinating field into which we must resist the temptation to enter now.

Against such a background one may get a new perspective of that peculiarly variable but strongly egocentric system of organic designs that has called itself *Homo sapiens*, occasionally with some justification. Originating as an ex-brachiating anthropoid ape and achieving bipedality and dexterity by developing certain marked positive and negative anisomerisms in his backbone, pelvis, feet and hands, man early began to make organic designs for himself.

Not that he was the first to do this, for birds had long made nests and squirrels had laid up a stock of food for the winter. That is why they had a neopallium

and a mind to respond *now* unconsciously to future events. Such a system was possible in a world of recurrent patterns, day and night, wind and rain, feast and famine, bark and bite. But after a prodigious anisomerism of the neopallium *Homo sapiens* far outstripped his fellow-mammals in the art of reading (and

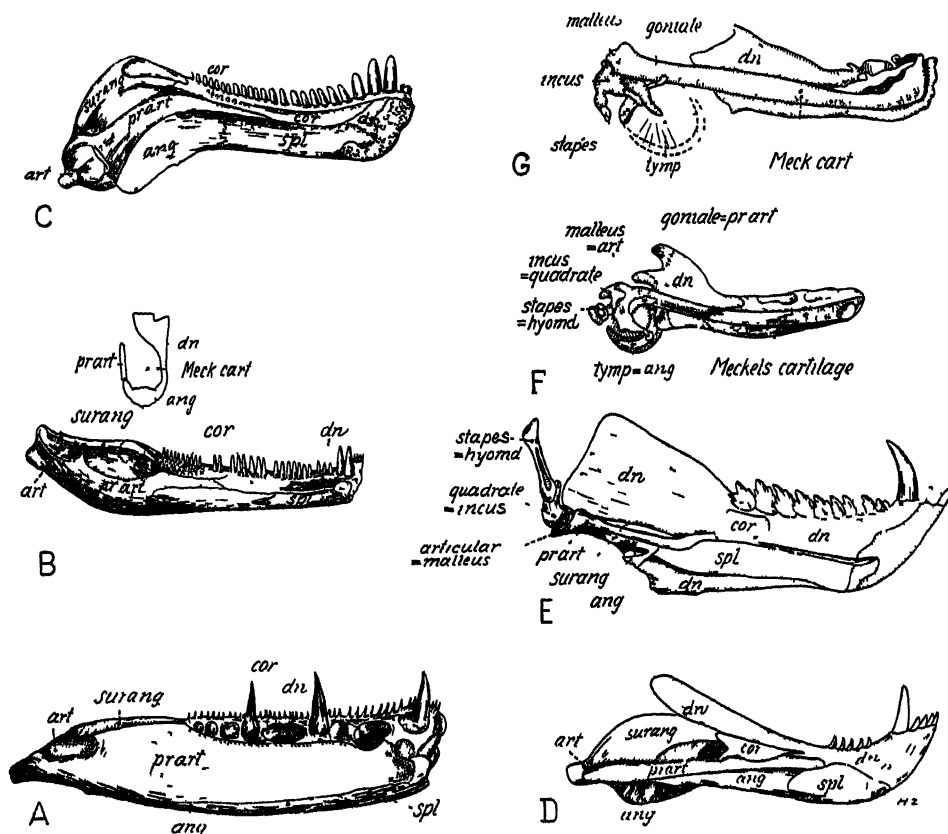


Fig. 10. Progressive positive anisomerism of dentary bone (dn) and negative anisomerism of bones behind dentary. Mesial aspect of left half of mandible: A. Crossopterygian, *Megaluhthys*. After Watson. B. Stegocephalian, *Trimerorhachis*. After Williston. C. Primitive American theromorph, *Sphenacodon*. After Williston. D. Primitive theriodont, *Cynarioides*. After Broom. E. Ascending ramus of dentary conspicuous: E. Progressive cynodont, *Cynognathus*. Ascending ramus of dentary very large, other elements reduced. Modified from Broili, by addition of quadrate and incus from other specimens. F. Foetal mammal, *Erinaceus*, showing relatively large size of malleus and incus. The malleus is formed from the proximal end of Meckel's cartilage. After W. K. Parker. G. Foetal man, showing connection of auditory ossicles with Meckel's cartilage. After Macklin.

misreading) the signs of past and future events. Especially when the habit of verbalised thinking had become fixed, conditioned responses were integrated on the one hand into constructive thinking and on the other into the *post hoc propter hoc*, *ab uno dice omnes* and other fallacies that have made *Homo sapiens* a mythophile with a gargantuan appetite.

VIII. SUMMARY

Paley argued that because natural mechanisms often work like human mechanisms they must have been made by a great designer; but this was an anthropomorphic fallacy which left the time element out of account. Darwin was able to show that in many cases intergrades are known that connect even the most complex natural mechanism with simpler antecedents; also his principle of the natural selection of heritable variations seemed to provide a mechanism for the production of mechanisms. However, the discovery of great numbers of what have been called orthogenetic series has obscured the principle that the natural selection of small heritable variations (mutations of the geneticists) conserves and integrates originally independent variables and tends to eliminate aberrant or "fortuitous" variations and lethals that lie too far to one side of the curve. That there is indeed a subsidiary mechanism for the elimination of the vast majority of merely random variations is suggested by the discovery of "organisers", which preside over the course of development and tend to keep it within prescribed limits (Spemann, 1927).

It is not, however, the purpose of the present article to discuss the causes of evolution but merely to formulate in general terms the ways in which organic designs of known history have originated and evolved, especially during the emergence and deployment of the vertebrates. An organic design is defined as a collocation of parts of an organic whole, varying in magnitude, emphasis or distance in space or time from the chosen point of origin or reference. As thus defined the changes in many typical organic designs of known history may all be expressed as the resultant of two co-operative principles of individual development and phylogenetic evolution: the first may most briefly be called *repetition*, the second *emphasis*. The principle of repetition has long been recognised in part under such names as "repetitive acceleration" (Cope), "metamerism" (Gegenbaur), "merism" (Bateson), "aristogenesis" (Osborn), while the principle of uneven development or emphasis has been called "differentiation" (Spencer), "alloiometry" (Osborn), "heterogony" (Pézar, J. S. Iluxley), and so forth. For some years past the writer has been calling the products of organic repetition *polyisomeres* and the general method of repetition, budding or reproduction *polyisomerism*; while for parts or regions that become unevenly developed, increased, decreased or fused with their neighbours the term *anisomeres* is employed and the process itself is called *anisomerism*. Now one and now the other of these processes may predominate, but both are constantly altering organic designs to a greater or less degree. The term *secondary polyisomerism* is used to denote the important fact that when one polyisomere acquires a certain detailed character its neighbours all along the line usually change in the same direction. This, as it were, throws a screen of small details over the surface and imparts an often false appearance of simplicity, homogeneity and primitiveness to what is in reality a very advanced stage of specialisation. When anisomeres are affected by secondary polyisomerism the pattern tends toward dedifferentiation.

After long periods of divergent evolution each of the diversified descendant genera of a common stock will be found to be in possession of: (a) a mask of changed

organic designs relating especially to its particular mode of life, which is collectively called its *habitus*, and (b) a smaller fund of unchanged organic designs or characteristics inherited from the remote common stock, the totality of such characters being called its *anatomic heritage*. Part of the *habitus* of a remote common ancestor after a change of function becomes part of the heritage of its descendants, and the two correlative terms may be qualified so as to indicate such conditions as the relative phyletic ages of any particular *habitus* and *heritage*, or the relative systematic values. Thus the swordfish-like *ordinal habitus* of ichthyosaurs dates from Triassic times, but their reptilian *class heritage* is of Permian age; the *family habitus* of the Macropodidae is kangaroo-like, but their *superfamily heritage* is phalangeroid. By extension of the same principle we may speak of the *habitus* and *heritage* features of some particular division of an organic design, such as the jaws and dentition, the feet, the reproductive system and the like.

Changes in *habitus* and *heritage*, if proceeding at slow rates or for relatively short periods, often convey the impression of orthogenesis, or undeviating evolution, but by combining the best established results of comparative anatomy and embryology with the records of palaeontology over long periods of geologic time we discover the reality and frequency of the phenomenon of *transformation*.

In reviewing the transformation of organic designs as seen in the deployment of the vertebrates, the writer holds that his method has on the whole not been *a priori*, since the foregoing principles were established objectively after the actual history of the vertebrates had been illuminated by the largely independent labours of a great number of geologists, palaeontologists and comparative anatomists, who for the most part were always interested in solving concrete historical problems rather than in considering the general principles of evolution. The principles of repetition and emphasis, of *habitus* and *heritage*, of undeviating evolution, transformation and the like, enable us to conceive the discrete facts of evolution in general terms, but these concepts to be effectively applied must naturally be supplementary to discovery of the historical facts.

With these provisos the writer has given a very brief review of the historic problem of the origin of the vertebrates, rejecting the claims of various phyla, such as the annelids, nemerteans, arthropods, etc., on different grounds and showing that astonishing changes among certain echinoderms from an essentially quinquerradial to a functionally bilateral symmetry warn us not to overlook the possibility that the invertebrate ancestors of the vertebrates may not have been bilaterally symmetrical, fusiform, free-swimming types but possibly sessile bottom-living forms with a strong dorso-ventral asymmetry.

The conclusions reached may be summarised as follows:

Amphioxus is secondarily polyisomorous in many respects and anisomorous in others, and may well be a degraded and highly specialised derivative of the oldest known chordates, the ostracoderms.

The contrasts are great between these earliest known chordates and any known invertebrates, with the possible exception of the carpioid echinoderms.

The most primitive of the ostracoderms appear to be among the Heterostraci,

especially the poraspids. These are fusiform fishes with an armour of five plates covering the head and front part of the thorax. In the thelodonts and coelolepids the shield is represented by a great number of small tubercles or scales which have arisen by secondary polyisomerism from a once continuous head shield (Kiaer).

The Heterostraci have paired nasal sacs, and according to Kiaer are related to the ancestors of the jaw-bearing fishes and higher vertebrates. From present evidence this relationship may well be much closer than would be possible under the older view that the heterostracous ostracoderms were a highly specialised side branch.

The gnathostomous or jaw-bearing vertebrates probably arose by transformation from agnathous creatures that bore no special resemblance to them except in the possession of certain "basic patents", especially the serial gill pouches.

With regard to the origin of the modern Agnatha (lampreys and hagfishes), the superb material and intensive investigations of Stensiö leave no doubt that the lampreys are derived from the cephalaspid ostracoderms, a result of great importance in the morphology of the central nervous system, since it confirms the conclusions of neurologists that the arrangements of the cranial nerves and general brain patterns of the larval lamprey are on the whole much more primitive than those of the elasmobranchs.

The investigations of Patten and Stensiö on *Bothriolepis* and of Stensiö on the Arthrodira have proved that these long extinct placoderms represented early offshoots from the base of the jaw-bearing series of classes. They seem to the present writer also to be derived eventually from some agnathous but branchiate ancestors who could not be very far from the most primitive of the heterostracous ostracoderms. Heintz's admirable work on the evolution of the Arthrodira from the acanthaspids has also provided a clear example of the interaction of polyisomerism and anisomerism.

The same principles are everywhere apparent in the recorded deployment of the higher vertebrates, as illustrated by the changes in the body form in the teleosts, or by the strange transformation of the mandible in the ancestry of the mammals.

Thus one may gain a new perspective on the origin of that peculiarly variable system of organic designs that has called itself *Homo sapiens*, who after a prodigious expansion of his neopallium began to make organic designs for himself; then, with growing pride and egotism and with all the prejudices of his race, nationality and profession, easily persuaded himself that he was a respectable even if much reduced copy of a "Great Designer".

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PHYSICO-MATHEMATICAL METHODS IN BIOLOGICAL SCIENCES

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I. INTRODUCTION: GENERAL CHARACTERISTICS OF THE PHYSICO-MATHEMATICAL METHOD

THE importance of physics in biology is at present generally recognised. But while physics in the twentieth century has developed into a rational mathematical science, biological sciences still on the whole must be classified among the purely empirical, descriptive sciences. A "mathematical biology", similar to mathematical physics, has not hitherto existed. In modern physics the most important discoveries are being made by mathematical calculations and theoretical reasoning. The leading physicists of to-day, such as Heisenberg, Einstein, Dirac, Schrodinger, to whom physics owes the greatest progress, have never done much experimenting, if any, thus giving proof of the almost inexhaustible powers of human thought. The biologist, however, still remains sceptical towards those mathematical methods.

This situation is easily explained. The increased specialisation in sciences makes it very difficult for one person to be thoroughly versed in two different sciences, such as physics and biology. The physicist has plenty of his own problems to work with. His work is to a large extent independent of the development of biological sciences, and as a result of this he usually knows next to nothing about them. The situation of the biologist is different. His work depends very much on the knowledge of physical phenomena. He is therefore more interested in physics than the physicist is interested in biology. But the necessity of absorbing the tremendous amount of empirical biological data consumes too much time to allow an adequately thorough study of physico-mathematical sciences. As a result of this the modern biologist's equipment in physics is in a sense not up to date. By this we do not mean

that he is not in possession of up-to-date facts. The difficulty is of a different nature. Whereas physics has already developed from the stage of a purely empirical, merely fact-seeking science, into a definitely rational science, biology as a younger science is itself passing through that pre-rational stage, and therefore quite naturally the biologist's frame of mind, even in his use of physics, is still purely empirical. Biophysics is becoming a sort of fad. But in this ultra-modern union of the two sciences, only the purely empirical side of physics is added to biology. And this at a time when, as we have said, the greatest progress of physics is being made through the application of rational, mathematical methods. The letter, the form of physics but not its spirit, is applied to biology.

There is another good reason for the sceptical attitude of biologists to mathematical theory. It lies in the human nature to condemn a remedy if it fails because it is applied in the wrong way. The same situation we find with respect to theoretical methods.

Besides knowledge, it takes also experience to make any good experimental work. This is realised by everybody. But it does not seem to be equally well realised that correct theorising also requires knowledge and experience to theorise. Nobody will blame the experimental method in principle if a young student gets some wrong results. This will be put down to his lack of ability in experimenting. But when attempts are made in biological sciences to theorise by people who have only a superficial idea of the theoretical science *par excellence*, mathematical physics, and when these attempts fail, then the mathematical method is considered as being at fault! Yet this same method in the hands of a Maxwell resulted in our enjoying listening to music over the radio, and this same method in the hands of Laue resulted in wholesale prevention of failures of important structural units in machines, by means of X-ray analysis!

It appears to be worth while to try the one thing hitherto not tried in biology, namely the building of a "system of mathematical biology", similar to mathematical physics. This task is not a small one, and one hardly could expect any spectacular achievements in a short time. It took two centuries of efforts of the best mathematicians to bring mathematical physics to its present perfection. Yet somebody has to start, no matter how difficult the task and how slow the progress. In the present article I shall give a very brief summary of what results have so far been obtained.

In our study, we should first start with the fundamental living unit—the cell. Following the fundamental method of physico-mathematical sciences, we do not attempt a mathematical description of a concrete cell, in all its complexity. We start with a study of highly idealised systems, which at first may not even have any counterpart in nature. This point must be particularly emphasised. The objection may be raised against such an approach that such systems have no connection with reality, and therefore any conclusions drawn about such idealised systems cannot be applied to real ones. Yet this is exactly what has been and always is done in physics. The physicist goes on studying in detail mathematically such non-real things as "material points", "absolutely rigid bodies", "ideal fluids", and so on.

There are no such things as those in nature. Yet the physicist not only studies them, but applies his conclusions to real things. And behold! Such an application leads to practical results, at least within certain limits. This is because within these limits the real things have common properties with the fictitious idealised ones. Only a superman could grasp mathematically at once all the complexity of a real thing. We, ordinary mortals, must be more modest and approach reality asymptotically, by gradual approximations. The failure of some of the above-mentioned "pseudo-theorising" has been due to the neglect of this fundamental rule.

II. MATHEMATICAL BIOPHYSICS OF CELLULAR GROWTH AND MULTIPLICATION

If we look for a *perfectly general* definition of a cell (R.¹ 1934*c*, 1935*e*), we find that it can be described as a small metabolising system, which grows as a result of its metabolism. By metabolism we mean quite generally the presence of some physico-chemical reactions which result in the transformation of some substances so that a group of substances is absorbed by the system from outside and another group is produced and diffuses out from the system. The nature of the reactions we shall at first leave indefinite.

At first glance not much can be done with such a general definition. Mathematical analysis, however, shows the contrary. Remembering what we just said about the study of over-simplified, idealised systems, we shall use the words "metabolising system" and "cell" indiscriminately, though some may object that what we call "cell" is not a real cell at all, and would perhaps be called best a "cell model". No harm is done by the use of our terminology, as long as we are aware of the abstraction which we are making. In developing the kinetic theory of gases, the physicist in the first approximation conceives the molecules as being very similar to minute rigid billiard balls and treats them as such. However, he does not speak of "aggregates of billiard balls", but of "molecular aggregates". Yet we now know that perhaps there is less difference between the abstract picture of the cell, which we are going to study, and a real cell, than between a billiard ball and an actual molecule. Just as in geometry, starting with a small number of rather simple and obvious postulates with which at first sight not much can be done, we gradually arrive step by step to the most complex theorems, unravelling to us various intricate relations in complicated geometrical figures, similarly in the present case mathematical analysis shows us that the simple concept of a metabolising system or cell contains in it many more implications than are seen at a glance.

The first property of any metabolising system which can be demonstrated mathematically is the following: the concentration of any of the substances which are either produced in the cell and diffuse outward, or are diffusing into the cell and are there transformed in some way, is not uniform either in the cell itself or in its surroundings. Regardless of the particular way in which a substance is produced and diffuses outward, its concentration will decrease inside the cell

¹ R. in this and subsequent references means Rashevsky.

towards the periphery; and outside the cell it decreases with increasing distance from the latter. On the contrary, the concentration of any substance diffusing into the cell decreases inside the latter as we proceed from the periphery to the central portion; and outside the cell it decreases as we approach the cell (R. 1931 *d, e*, 1934*b*). The exact manner of variation of the concentrations inside and outside the cell varies from case to case, and is determined by the shape of the cell, by the physical properties of the cell and of the surrounding medium, and by the nature of the physico-chemical reactions which produce or consume the substances. But the existence of non-uniformities is quite general; it follows with mathematical certainty from the fact of metabolism. As soon, however, as the metabolism stops for one reason or another, the gradients disappear.

The next step is to study the consequences of these non-uniformities, or as the physicist calls them, gradients of concentrations. It is known that any dissolved substance produces an osmotic pressure, which varies with the concentration of the dissolved substance. For low concentrations within a considerable range, the osmotic pressure is proportional to the concentration. For high concentrations the variation of osmotic pressure with the concentration is somewhat more complicated. Combining the existence of osmotic pressure with the existence of gradients of concentrations in and outside any metabolising system, we arrive at the conclusion that both inside as well as outside any metabolising system the osmotic pressure is not uniform, but varies from point to point. The next step is made by using a fundamental theorem of physics, which states that any system in which the pressure (osmotic, or of any other origin) is non-uniform is the seat of mechanical forces, the distribution of which can be calculated from the distribution of the pressure.

Hence we arrive at the inevitable conclusion, that every metabolising system, regardless of its particular characteristics, and therefore every metabolising living cell, is the seat of a more or less complex system of mechanical forces. Those forces, their magnitude and distribution, are determined by the distribution of osmotic pressures. The latter in their turn are determined by the distribution of concentrations. And those finally, as we have seen, vary from case to case, being determined by the size, shape and physico-chemical properties of the system. Hence those particular characteristics of the system will also determine the forces. Our next step is therefore clearly indicated. We must systematically investigate various possible cases, beginning with the simplest ones, and calculate for each case the forces. Knowing these we shall be able to calculate their possible effects on the cell.

Before proceeding to such a systematic study, we must, however, point out that concentration gradients produce mechanical forces also in a different way from that outlined above and connected with the existence of osmotic pressure.

We know from physics that molecules of any substance are complex dynamic systems, and that any two molecules of the same or different kinds, either attract or repel one another. Such forces of attraction or repulsion exist also between the molecules of a solvent and a solute. If the latter is distributed uniformly, the resulting force on any molecule of the solvent is zero. But if the dissolved substance

is distributed non-uniformly, so that there are more dissolved molecules on one side of a molecule of the solvent than on the other, this asymmetry results in a force acting on each molecule of the solvent, and therefore on each element of volume of the latter. We thus see that even regardless of the existence of osmotic forces, concentration gradients produce forces, due to molecular attraction and repulsion (R. 1934 *b, d*).

The mechanical force produced by non-uniformity of osmotic pressure is a function of temperature. For the case when the osmotic pressure p is proportional to the concentration c of the dissolved substance, the force acting on each element of volume is equal per cubic centimeter to

$$f_0 = -\frac{RT}{M} \text{grad } c, \quad \dots\dots(1)$$

where R is the constant of the well-known equation of perfect gases :

$$pv = RT,$$

and is equal to 0.8×10^8 erg degree $^{-1}$ mol $^{-1}$, T being the absolute temperature, and M the molecular weight of the dissolved substance. As for the symbol grad c , it denotes the rate of change of the concentration at a given point in the direction of the most rapid variation. If, for instance, around any point the concentration varies only in one direction, in which we shall lay the x -axis of a system of co-ordinates, then grad c is the same thing as dc/dx . The minus sign in (1) indicates that the force acts in the direction of decreasing concentrations (direction from higher to lower osmotic pressure).

The force due to molecular interaction is given (R. 1935 *d*) by

$$f_m = -\frac{L}{2M} \text{grad } c, \quad \dots\dots(2)$$

where L denotes the work necessary to bring a grammol of the solute from a state of perfect gas into the solution. This work is positive when the molecules of the solvent repel those of the solute, and is negative when they attract. Hence in the case of attracting forces between molecules, the mechanical force f_m is directed towards regions of increasing concentrations, being thus opposed to the osmotic forces. For repelling forces between molecules, both f_0 and f_m have the same direction.

L is closely connected with the heat of solution as well as with some other physico-chemical constants, which are directly measurable. It can therefore be calculated, and the magnitude of the factor RT in (1) compared with that of $L/2$ in (2). Such a comparison (R. 1934 *b, d*, 1935 *d*) shows that f_0 and f_m are of comparable magnitude, although f_0 is generally larger than f_m , so that the total mechanical force is usually directed towards decreasing concentrations.

Now we proceed to the study of various possible cases of metabolising systems. As regards shape, we choose the simplest one—that of a sphere. Many actual cells are very nearly spherical. But with some approximation our formulae will apply also to cells which are not exactly spherical, as long as the deviation from the spherical shape is not excessive.

As to the physical constants of the system, we consider first the simplest case of a physically homogeneous system, characterised by a diffusion coefficient D_i for a given substance. The external medium we shall also consider as homogeneous, with a diffusion coefficient D_e . The membrane of the cell we take as being characterised by a permeability h , which is defined as the amount of substance passing per minute per cm^2 of the cell surface, when the difference of concentrations on both sides of the membrane equals 1 gr. cm.^{-3} . And finally we begin by considering the simplified, actually non-existing case of *one* substance only being metabolised, at a constant rate $q \text{ gr. cm.}^{-3} \text{ min.}^{-1}$. If a substance is produced at such a rate, and diffuses outward, then the distribution of concentration inside (c_i) and outside (c_e) possesses spherical symmetry, and is given (R. 1932*b*) at any point whose distance from the center is r , by:

$$\left. \begin{aligned} c_i &= c_0 + qr_0^2/3h + qr_0^2/3D_e + q(r_0^2 - r^2)/6D_i, \\ c_e &= c_0 + (qr_0^2/3D_e)(1/r), \end{aligned} \right\} \quad \dots\dots(3)$$

in which r_0 denotes the radius of the cell, and c_0 the concentration of the substance in the medium in the absence of the cell. In particular c_0 may be zero in this case. Equation (3) shows that c decreases from center to periphery, and at sufficiently large distances becomes equal to c_0 .

If we consider the case of a substance, diffusing *into* the cell and consumed there at a rate q , we shall find the same expressions, except that the sign of q must be taken negative ($-$). The concentrations now *decrease* as r *decreases*. It is also clear that c_0 cannot be zero; for in order that a substance should diffuse constantly into the cell, there must be a supply of it in the external medium. As a matter of fact, for equation (3) to hold in that case, c_0 cannot fall under a critical value c^* . The first of equations (3) shows that as c_0 decreases the concentration at the center of the cell for $r=0$ will also decrease, until for a certain value $c_0=c^*$ it becomes zero. For still smaller values of c_0 the substance does not penetrate to the center of the cell. It is consumed only inside a spherical zone, whose outer radius is r_0 and whose inner radius r_1 increases with decreasing c_0 , becoming equal to r_0 for $c_0=0$. For this value of c_0 the zone becomes infinitely thin. Since we consider the case of a constant rate of reaction *per unit volume*, it is clear that as the volume of the zone in which the consumption takes place decreases, the *total* rate of consumption for the whole cell will also decrease, approaching zero for $c_0=0$. This circumstance has been taken by Gerard (1931) to account for the gradual decrease of oxygen consumption of some eggs with decreasing oxygen concentrations in the surrounding medium. A mathematical treatment by Gerard shows that estimates of the values of D_i can be made from observations of variation of oxygen consumption with oxygen pressure. A more general mathematical treatment by Rashevsky (1933*c*) shows that not only D_i , but also h , can be calculated from such observations. From Gerard and Tang's (1932) data on fertilised *Arbacia* eggs, Rashevsky (1933*c*) finds for oxygen:

$$D_i = 7 \times 10^{-7} \text{ cm.}^2 \text{ min.}^{-1}; \quad h = 4.25 \times 10^{-4} \text{ cm. min.}^{-1}. \quad \dots\dots(4)$$

In some cases, however, experimental data cannot be represented by the equations developed. This is most likely due to the circumstance that the rate of consumption of oxygen is not independent of other reactions going on in the cell at the same time. We shall discuss this question below. In view of this limitation, the values (4) cannot be considered as exact. They give us, however, an estimate of the order of magnitude of D and h .

As a next case we may consider a cell which produces or absorbs a substance at a rate which is proportional to the concentration of the substance produced or consumed. Much more complicated expressions are obtained in this case (R. 1931*d*), but the general character of the concentration distribution remains the same. For produced substances the concentration decreases with increasing distance from the center, for consumed substances the reverse is true.

Still more complex equations are obtained when we calculate the distribution of concentrations in a system consisting of two phases arranged concentrically. Such a system comes closer to actual cells with their nucleus. The general characteristic of the gradients, however, still remains the same.

When a substance is produced it is essential, however, that it should diffuse outwards. If the cell membrane is impermeable to a substance produced, so that it accumulates inside the cell, its concentration will be uniform, and it will not contribute to the production of the forces discussed above.

An important remark must be made at this point. When an arbitrary initial distribution of concentrations is given in a metabolising system, it varies in general with time, approaching asymptotically a stationary distribution given by equation (3) or other equations just mentioned. In order that the above equations could be applied, it is necessary that during the time which is required by the system to reach the stationary state, the size of the system should not appreciably vary (R. 1928*b*, 1931*d*). An estimation of this time shows it to be of the order of magnitude of a few minutes, and since the size of a cell does not vary appreciably during such a short time, the use of those equations is justified.

Having calculated the distribution of concentrations in various cases, we may now proceed to calculate the forces which they cause. Remembering that those forces are directed from points of higher concentration towards points of lower concentration, we see that a produced substance will generate forces which will be directed from the center to the periphery, whereas consumed substances will generate forces directed inwards. Again, consider first the idealised case of a system metabolising only one substance. If this substance is produced by the system, the resulting forces are directed so as to have a tendency to tear the system apart. If the substance is consumed by the system, then the resulting forces are directed so as to compress the system. If we consider a system, having the shape of a perfect sphere, in either case the forces will be distributed symmetrically around the center, and they will not produce any deformation of the spherical shape. But there are no perfect spheres in nature. And any deviation from spherical form will result in a deviation of the distribution of the forces from perfect spherical symmetry. The mathematical study of such a deviation amounts to the calculation of the distribution

of concentrations in a system which is almost, but not exactly, spherical. Then, from the distribution of concentrations, the distribution of forces is calculated. This rather complex mathematical problem has been treated for various cases (R. 1934*d*, 1935*c*). The results can be summarised as follows.

When the system which deviates slightly from the spherical shape produces a substance, the forces are distributed so as to tend to elongate the system and eventually divide it in two. When such a system consumes a substance, the forces tend to bring it as close to a spherical shape as possible. A system which produces a substance will therefore spontaneously divide in two, and such division would proceed indefinitely, if no opposing forces are present. Such an opposing force is always produced by the surface tension, which counteracts any dispersion. Calculation shows that for small sizes of the cell the forces due to surface tension exceed the forces due to metabolism. A spontaneous division cannot therefore occur. But above a critical size the metabolic forces prevail, and the system divides spontaneously. If such a system increases in size through growth, it will divide when it exceeds the critical size. The two "daughter systems" will grow farther, until they divide, etc. For the case of a substance produced at a constant rate q in a single-phase spherical cell, the critical radius above which the cell divides spontaneously, no matter how small its deviation from spherical shape, is given by

$$r_c = \sqrt[3]{\frac{12\gamma}{q[(RT/\bar{M} - \Gamma')/D_e - (\Gamma - \Gamma')/D_i]}} \quad \dots\dots(5)$$

where $\Gamma = \frac{L}{2\bar{M}}$ and Γ' are similar constants, relating to the substance of the cell membrane, and γ is the surface tension of the cell surface.

A similar formula is obtained (R. 1935*c*) for a cell consisting of two concentric separate phases, analogous to cytoplasm and nucleus.

A system which only consumes a substance will of course never divide spontaneously, for both metabolic and capillary forces oppose division.

If we wish to apply our calculations to actual cells, we must remember that every cell both produces and consumes a great number of substances. The forces caused by produced substances tend to divide the cell, those caused by consumed substances counteract that tendency. In order to calculate the resultant effect, we must take the sum of all "dividing" forces, and the sum of all "stabilising" forces, and see which prevail. The rather meager knowledge of all rates of reactions and constants involved would make such a problem hopeless. Matters, however, are considerably simplified by the following consideration: the equation for the forces, which are obtained by combining equations (1) and (2) with equation (3), or other similar equations, show that the force is proportional to the rate of reaction q of the substance which generates the force. In taking the sum of all the forces produced by the many substances involved in actual cellular metabolism, we may therefore neglect those substances which have a very small q . Fortunately, a survey of cellular metabolism shows that all reactions fall into two distinct groups as regards their rate. The reactions involved in respiration, namely, consumption of

sugar, production of lactic acid, consumption of oxygen, production of carbon dioxide and water, proceed at a rate of the order of magnitude of 10^{-4} gr. cm. $^{-3}$ min. $^{-1}$ (R. 1932c), whereas other important reactions, such as deamination of proteins, etc., proceed at a rate of 10^{-7} gr. cm. $^{-3}$ min. $^{-1}$, or even less. That is, they are a thousand times slower. Forces due to the second group of reactions will be much smaller than those due to the first group. In other words, *as a first approximation*, we may consider only the first group of reactions. The task of calculating their effect is now not so hopeless, and has been carried out with the following result (R. 1935d).

When oxidation of sugar is complete and lactic acid is not produced, the resulting forces are such as to inhibit division. With a sufficiently high glycolytic coefficient, the dividing forces prevail. The stronger the glycolysis, and the less complete the final oxidation relative to the glycolytic fermentation, the stronger the tendency of the cell to divide. Calculation of the absolute value of the forces, and of the critical sizes at which division occurs, shows that the latter are of the order of magnitude of 3×10^{-3} cm., *which is the average size of actual cells*.

The objection may be raised that all the above theory would fail in principle in those cells in which protoplasmic streaming is observed, because such streaming would abolish the non-uniformities of concentrations. Rashevsky (1934b, 1935e) shows, however, that this is based on a fallacy. The streaming undoubtedly *disturbs* the normal gradients, but does not destroy them. Actually the existence of streaming is a direct proof of the existence of gradients. Any streaming can only be due to mechanical forces, and in a perfectly homogeneous system no such forces can exist. No gradients, no forces; no forces, no streaming. The special types of concentration distribution are, however, naturally changed by streaming, and a mathematical investigation of such cases is still wanting.

The forces producing division are directed, as we have seen, outward and tend to expand the system, increasing its size, if this is compatible with other physico-chemical conditions. They are therefore a contributing factor to *growth* (R. 1935h). From what has just been said, abnormal glycolysis results in both abnormal growth and abnormal rate of division. This is in agreement with our present knowledge of the metabolism of tumors (Warburg, 1930).

Thus, starting with a rather general conception of a metabolising system, and having investigated mathematically a number of purely abstract cases, such as systems metabolising only one substance, etc., we finally arrive at a synthesis of our results, which brings us into direct contact with actual facts about growth and multiplication in their relation to metabolism, and throws interesting light on hitherto purely empirical relations.

Our next step is to complicate our theoretical system still further, bringing it still closer to actual cells. This has been done in two ways. First by studying further effects of the forces due to metabolism, second by abandoning the simplified assumption that the several reactions which take place in a cell are proceeding at constant rates, independent of each other.

We have discussed the forces which are exerted by the molecules of the solute on each molecule of the solvent. Similar considerations show that the molecules

of a dissolved substance will exert forces on molecules of other dissolved substances or on colloidal particles present in the system. Calculations show (R. 1935*h*) that the effects of those forces on other *molecules* is negligible, but that their effect on larger molecular complexes, colloidal particles, whose size exceeds 10^{-5} cm., may be very strong, resulting in a concentration of those particles either in the center or at the surface of the cell. Thus non-uniformities of a structural nature are produced by non-uniformities of concentrations, and those structural non-uniformities affect the physical constants of the system, in particular its permeability. It is clear that an aggregation of colloidal particles of any nature near a surface will in some way affect the permeability of the latter. Since the forces which produce such structural changes exist only as long as the system metabolises, the death of the cell will result in a sudden rearrangement of the colloidal constituents, which will in its turn produce a sudden change of permeability (R. 1935*h*). The sudden changes of permeability at death is a fact well known in biology.

The permeability of a cell cannot be considered any more as a constant, but is itself to a large extent determined by the metabolism of the cell. Considering now possible interrelations between the various fundamental metabolic reactions, taking, for instance, into consideration that the oxygen consumption is determined partly by the amount of lactic acid to be oxidised, the amount of the latter depending on the concentration of the glucose, etc., and combining this with the above results, we arrive at a rather complex picture of a cell. The mathematical treatment of the problem becomes more difficult, but the results compensate for the difficulties. Again, a number of possible cases must be studied systematically. We shall mention here only one case of great interest. A complex system of such a nature possesses the following interesting property (R. 1935*h*). It is capable of two distinct physico-chemical configurations. One is characterised by low permeability and low glycolytic coefficient; hence in this state the system either does not grow and divide at all, or does so at a low rate. The second configuration is characterised by a high permeability and high glycolytic coefficient; hence rapid growth and division. The system can be brought irreversibly from the first state into the second by a temporary inhibition of oxidation. The importance of this result for the understanding of the well-known experiments of Warburg (1930) on producing cancer-like growth by temporary cyanide asphyxiation, as well as of the reported observations on the higher permeability of cancer cells (Fisher, 1927), is obvious.

Mathematically such a complex system is characterised by a large number of physico-chemical constants. The next problem is to study the effect of the variation of those constants on the transition of the system from one state to another. A way is thus indicated to a mathematical investigation of possible methods of prevention and treatment of cancer. The way is certainly a long and difficult one, but not impracticable. Years ago, when all efforts of astronomers to locate a particular asteroid, lost amongst hundreds of others, failed, Gauss by mathematical calculation alone found the place where the asteroid was to be looked for, and thus made possible its rediscovery. Who knows whether mathematical analysis is not destined to play a similar role in the study of one of the most important biological problems,

when the efforts of many experimenters have failed, because the factor or factors they are looking for is lost amongst hundreds of others?

So far we have considered the effects of such metabolites, the molecules of which do not carry any electric charges. The next step is to investigate the metabolism of strongly ionised substances. The appearance of electric charges in disperse systems is a well-known phenomenon. These charges are produced either by selective adsorption of ions, or by selective solubility, or else by selective permeability (Donnan equilibrium). All these factors are of a "static" nature independent of any possible physico-chemical reactions. It is, however, important that when a system metabolises different ions this results, due to differences in the diffusibility and permeability for various ions, in an appearance of electric charges, which are quite independent of any of the above-mentioned "static" factors (R. 1935*a*). Even in the complete absence of those static factors those charges would appear. But they will last only as long as the system metabolises. With the cessation of metabolism, with the death of the system, these charges disappear. Here we have another interesting point in connection with sudden changes of electric charges at the death of a cell. These studies also throw some light on the effect of death on differential permeability to ions (R. 1935*h*).

The question arises as to how the electric forces due to the charges will effect the mechanism of division. A complete investigation is still wanting, but a preliminary investigation (R. 1934*b*, and unpublished results) shows that though these electric forces may have a pronounced effect on the dynamics of division, they will not affect it very materially, and that in the first approximation the neglect of these forces is justified.

It must be emphasised that the above-mentioned metabolic forces are by no means to be considered as the *only* factors that produce cell division, though they are the principal ones. Equations (3), and other similar ones mentioned above, show that the concentration of various metabolised substances near the surface varies with the size of the cell. This in its turn results in a variation of surface tension, and such a variation may be a contributing factor to spontaneous division (R. 1928*a, b*, 1931*d, e*, 1932*a*). Still another factor is found in considering surface adsorption and reversible reactions between the physico-chemical components of the surface phase and the volume phase of the system (R. 1928*c*).

In the earlier publications Rashevsky (1932*c, d*) used purely thermodynamical considerations (energy balance) to establish the conditions of spontaneous division. Cell sizes calculated in this way are also of the correct order. A more detailed study shows, however, that such a method is not sufficient for an adequate treatment of the problem (R. 1934*b, d*), and it has been superseded by the above-discussed studies of mechanical forces and their effects.

From the study of the simplest case of spherical cells we now pass to the investigation of non-spherical systems. It is a fundamental theorem of physics that a liquid system, when not subject to gravity or any other external forces, assumes the shape of a sphere, due to the surface tension effect. If, however, the liquid system is a seat of metabolic reactions, then the metabolic forces come into

play, and the spherical shape is not the only possible equilibrium configuration (R. 1929 *d, e*, 1931 *e*). However, such non-spherical shapes are possible only as long as the system metabolises. When metabolism stops, concentration gradients and the forces which they produce disappear, and the system returns to a spherical shape under the influence of surface tension. Many free cells which possess non-spherical shape during life (*Euglena*) round up after death.

III. CELLULAR AGGREGATES

From the study of single cells we now pass to the study of cellular aggregates. We have seen that concentration gradients exist not only inside the cell but also in the surrounding medium. The latter is therefore also a seat of mechanical forces, which are exerted both on every element of volume of the medium itself and on any foreign body included in the medium. Therefore a metabolising cell will exert a force on another cell located in its neighborhood. This force is due to the inequality of osmotic pressures on two sides of the second cell. It may be either a force of repulsion or a force of attraction, depending on the distribution of concentrations (R. 1934*b*). Besides this osmotic force, a force of atomic origin also in general exists between two metabolising cells (R. 1932*b*). The magnitudes of both types of forces have been estimated and found to be quite strong at close range. Per cell of the size of 10^{-3} cm. they are of the order of 10^{-2} dyn., which, expressed per cm^3 , gives a force of 1 kg.

A correlation exists between the character of the forces between cells, and those studied previously. Cells in which "dividing" forces prevail in general repel each other. Cells which do not divide attract each other (R. 1934*b*). Since functional nerve cells do not divide at all, we would expect particularly strong forces of attraction between them. This has been suggested to account for the formation of ganglia, especially during the early embryonic stages, when the cells are relatively easily displaced with respect to each other (R. 1933*d*). The peculiar branched irregular form of neurones can be also derived on this basis (R. 1933*d*). On the other hand the tendency of cancer cells to form metastases, their relative "looseness", is in agreement with the conclusion that rapidly dividing cells should repel each other.

Considering an aggregate of cells of different characteristics, one is led to the study of the arrangement which they will assume under the influence of those forces. The exact mathematical treatment of the problem is very difficult and is still wanting. But some general considerations indicate the possibility of an approach on this basis to a physico-chemical theory of the morphology of Metazoa (R. 1933*d*), and of a mathematical interpretation of general embryology.

The problem of simpler cellular aggregates may also be approached in a somewhat different way, namely by considering a system with many phases and a very large specific surface, so that the surface energy is by no means negligible as compared with the total energy (R. 1929*b*, 1931*d*). Such systems possess very interesting properties. Under some rather general conditions they exhibit pheno-

mena of differentiation, increasing in complexity when increasing in mass. Parts of them, separated from the whole system, regenerate into complete systems, etc.

IV. PHYSICO-MATHEMATICAL THEORY OF EXCITATION AND NERVE CONDUCTION

A field of general biology in which a physico-mathematical approach has been attempted earlier than in any other fields of this science is that of excitability. A systematic, deductive theory of this class of phenomena is still wanting. In this field attempts have been made to work out theories and formulae immediately applicable to definite empirical data, instead of investigation *in abstracto* of various over-simplified possible cases without immediate concern about their applicability (R. 1934*a*), as is the method of mathematical physics. Perhaps this attitude is partly responsible for the lack of a very deep insight into the general nature of the phenomena of irritability. There are no royal roads in mathematical sciences, and an attempt at a short-cut usually results in a waste of time.

The mathematical approach of Weiss (1901) was of a more empirical nature and lacked the physical point of view. This physical point of view is found somewhat more in the work of Hoorweg (1892, 1893), though it still remains largely phenomenological. It is Nernst (1899, 1903) who first put the theory of excitability on a physical basis by introducing the conception that excitation was in all cases due to changes in concentration of exciting ions, produced by the electric current. This line has been followed by A. V. Hill (1910, cf. also a comment on that work by R. 1930*f*), Umrath (1924), and in a less detailed but somewhat more systematic form by Lazareff (1928). In all these papers attempts are made to represent a set of more or less complex phenomena, and from the start rather complex physical assumptions, introduced mostly *ad hoc*, are treated mathematically. The same tendency is shown in the subsequent development of the phenomenological theories, except that the complications here are purely formal, mathematical (Lapique, 1926; Ozorio de Almeida, 1926).

If one would follow the spirit of the physico-mathematical method, as has been done in the case of cellular biology, one would have to investigate the case of a simplest variation of the concentration of an ionised substance, with respect to time and space, under the action of an electric current, under as general conditions as possible. Such variations of concentration are represented by a partial differential equation, and the exact solution of the latter requires *definite special assumptions* about the configuration of the system. But as a first approximation we may consider the variation of concentration in a very small region, in which the variations with respect to space are negligible. Then we have to consider only the variation with respect to time, and the partial differential equation degenerates into an ordinary one. The simplest general form of such an equation is then clearly indicated, namely that of a first order linear equation. Such an approach has been recently made by Blair (1932 *a, b, c, d*, 1935 *a, b*), who in a series of papers has shown that this very general and yet simple equation accounts very well quantitatively for a large number of experimental data.

The next generalisation of the picture consists in the consideration of interaction of two or more ionised substances, all affected by the electric current. The antagonistic effects of the mono- and bivalent cations is well known, the latter inhibiting the excitation caused by the former. The simplest case is that of two types of ions, one excitatory, the other inhibitory. Rashevsky (1933*b*) has shown that this generalised picture not only includes all the results of Blair's theory, which has to be considered as a first approximation of this more general one, but also accounts in a natural way for the phenomenon of excitation at the anode on opening the current, which has been the stumbling block of all previous physico-mathematical attempts.

An important problem in the theory of excitation is the theory of its propagation. It is now generally accepted, especially since the classical experiments of R. S. Lillie (1923, 1925, 1936¹) on the passive iron model of the nerve, that conduction occurs through excitation of adjacent regions of the nerve by the local bio-electric currents generated in the already excited region. An elementary mathematical treatment of this concept has been attempted by Cremer (1924, 1929). By an exact mathematical investigation Rashevsky (1931*c*, 1933*a*) found that such a process of propagation is governed by entirely different mathematical equations to any process of propagation hitherto studied in physics. The law of propagation depends on the law of excitation, which we accept. The propagation is not always necessarily uniform, nor proceeds indefinitely. Thus Hoorweg's formula was shown by Rashevsky (1931*c*) to lead to a continuously decreasing velocity, and the excitation cannot spread beyond a certain distance. Rashevsky (1933*a*) has investigated the law of propagation, taking as a basis Blair's (1932*a*) formula of excitation, and has derived for the velocity of propagation the expression

$$v = (I - R) k / \alpha R, \quad \dots\dots(6)$$

where I is the peak value of the action current per fiber, R the rheobase of a fiber, k a constant related to the chronaxie, and α a constant involving the radius of the fiber and its electrical conductivity. Blair (1934) has analysed available data and found equation (6) to be in agreement with them. Rashevsky (1935*g*) has also investigated the law of propagation on the basis of his generalised theory of excitation (R. 1933*b*) and arrives again at equation (6), except for very minute deviations to be expected in the immediate neighborhood of the original place of excitation.

V. PHYSICO-MATHEMATICAL METHODS IN PSYCHOLOGY

An application of physico-mathematical methods to the study of the central nervous system and of the highest brain activity has also been made. Two ways of approach have been used here. One of them is based on the thermodynamical study of complex heterogeneous systems. In this method of study such quantities as entropy or free energy are calculated in terms of the parameters which determine the configuration of the system, and then such values of these parameters are

¹ *Biological Reviews*.

determined as bring the corresponding quantities to an extremum; that is, such as give a maximum for the entropy or a minimum for the free energy. In this way the configuration of the system is determined.

A very usual case in such complicated systems is that the entropy or free energy possesses not one maximum or minimum but several. Physically this means that the system has several configurations of equilibria, and that therefore the specification of the environmental conditions (such as temperature, pressure or anything else that affects the system) does not specify the configuration of the system. Such cases are not unknown to ordinary thermodynamics, but there they are considered exceptional and have not been thoroughly investigated.

A general mathematical study of such systems shows that they are all characterised by one important property: they all exhibit what is known as hysteresis (R. 1929*a*). This means that the configuration of such systems at a given moment is not determined by the environmental conditions at that moment, but by the previous "history" of the system. Such phenomena as magnetic, colloidal and elastic hysteresis are only simple cases of more general hysteresis, and can be traced also to the existence of several equilibria in molecular and atomic configurations.

The same change in environment will cause different changes in the system, depending on the configuration which this system possesses. Speaking in more physiological terms, the reactions of such a system to the same environmental change will vary; they will depend on its "history", or, to be still more anthropomorphic, on its "previous experience". In a formal way this, however, is a characteristic of the behavior of all organisms, particularly of the "higher" ones endowed with a highly developed brain. This dependence of reaction on previous experience we attribute to learning. And, from a purely formal point of view, learning is nothing more than a particular kind of hysteresis (R. 1931*a*).

Taking the conditioned reflex as a particularly fundamental problem of the theory of the central nervous system, it may be observed that from a purely formal point of view the conditioned reflex may be described as a kind of hysteresis, possessing the following characteristics.

The variation of an "environmental parameter" α does not produce any change in the system. The variation of another "environmental parameter" β produces a definite change in the system. After α and β are changed simultaneously several times, the variation of α alone produces the same change as the variation of β .

By developing a general thermodynamical theory of such systems with hysteresis, a formal account of the fundamental phases of conditioned reflexes including differential conditioning not only to individual simple stimuli, but to complex combinations of the latter has been given (R. 1930*a*). An investigation of the details of the atomic mechanism of systems possessing several thermodynamical equilibria (R. 1930*c, d, e*) leads to a more physical picture of a colloidal mechanism, which exhibits various phenomena of learning and even more complex phenomena of "Gestalt-discrimination" (R. 1930*g*, 1931*a, b*). The physico-chemical structure of these "models of the brain" is too complex to permit a brief description within the limits of a review.

The second method of approach to the physico-mathematical study of the brain is by way of a generalisation of the findings in the mathematical biophysics of cells and peripheral nerves. The theory of forces of attraction between neurones leads us to a general interpretation of the phenomena described under different names, such as "neurobiotaxis" (Kappers, 1917), neurotropism (Ramon y Cajal, 1911), etc. A mathematical study of the possibility of formation of actually new interneuronic connections in this way leads to a general theory of conditioning and learning (R. 1935*f*). Furthermore, Rashevsky (1934*c*, 1935*b*) has shown that a generalisation of his theory of peripheral excitation is sufficient to account for the production of the *same* reaction by complex stimulus pattern, which may involve entirely different peripheral elements, as long as certain elements of order are preserved between the elements of the pattern. (For instance, recognition of a square as such, regardless of the position of its image on the retina, or its size.) These results bring the important problems of "Gestalt-transposition" into the domain of physico-mathematical investigation. A further generalisation leads to a physico-mathematical approach to the problem of behaviour (R. 1935*f*).

VI. SUMMARY

1. The fundamental method of exact physico-mathematical sciences, that of abstraction and of a systematic study of abstract, idealised cases, is outlined and the timeliness of its application to biology indicated.

2. This method is applicable to the study of general biology. The most general property of all cells being metabolism, the mathematical study of metabolising systems in general is indicated.

First it is shown that regardless of the special character of metabolic reactions in and around any metabolising system, the concentrations of the various substances involved in metabolism is not uniform, the non-uniformities being determined by the rates of reactions, size and shape of the system, etc.

Making use of the general physical laws connecting the concentration of a dissolved substance with the osmotic pressure, the conclusion is reached that in and around any metabolising system the distribution of osmotic pressure is not uniform.

Applying next a fundamental theorem of mechanics, we find that therefore any metabolising system is the seat of mechanical forces, the distribution of which is determined by the rate and type of reactions and other factors. A further mathematical study of the effects of non-uniformities of concentration shows also that the osmotic pressure is not the only factor that produces mechanical forces. Intermolecular attractions and repulsions also result, in cases of non-uniform concentration, in mechanical forces acting on each element of volume of the system.

A closer consideration of the system of forces thus produced by metabolism shows that, for substances produced in the cell and diffusing outwards, these forces are generally also directed outwards. One of their effects is the tendency to expand the system, and to contribute to its growth. For substances diffusing into the cell

and consumed there, the forces have in general the opposite direction and inhibit the growth of the system.

A detailed mathematical investigation of the other effects of those forces shows that, for the case of produced substances, those forces cause a spontaneous division of the system when the latter exceeds a critical size. Calculations of this size gives values identical with the average size of actual cells. The forces due to consumed substances inhibit spontaneous division.

In a system which, like an actual cell, produces and consumes a great number of substances, the effect will depend on which type of forces prevail. Calculations show that forces produced by reactions connected with cell respiration considerably exceed the forces due to all other reactions. In the first approximation therefore only respiratory reactions may be considered. Mathematical analysis shows that when oxydation of sugar is complete and no appreciable amount of lactic acid is formed, the forces inhibiting growth and division prevail. When glycolysis is strong, the forces which produce division and accelerate growth prevail. This is pointed out to be in agreement with O. Warburg's findings that abnormally growing and dividing tumor cells have an abnormally high glycolytic coefficient.

A further study of other possible effects of the forces produced by metabolism shows that they also will in general affect the permeability of the cell. Since the forces exist only as long as the cell metabolises, the death of the cell must result in sudden change of permeability, as is actually the case. The study of still more complex cases shows that the cell may possess two configurations of equilibrium. One is characterised by relatively low permeability and low glycolysis, hence by low rate of growth and multiplication. The other is characterised by a higher permeability and high glycolysis, hence rapid growth and multiplication. The cell, in such a case, can be brought irreversibly from the first configuration into the second, by temporary asphyxiation. This again is in agreement with Warburg's experiments on production of tumor-like growth by asphyxiation.

3. The non-uniformities of concentrations, and therefore the forces, are present not only within the cell, but also outside it. This results in forces of repulsion and attraction between cells. Cells in which the dividing forces prevail usually repel each other. Cells in which the inhibiting forces prevail, attract. The mathematical theory of the configurations assumed by cellular aggregates under the influence of such forces indicates a way to a physico-mathematical theory of organic forms of metazoans.

4. A physico-mathematical theory of nerve excitation and nerve conduction accounts for a number of empirical data. The generalisation of the ionic theory of excitation to the case of two types of ions, one exciting, the other inhibiting, gives a natural explanation for the excitation at the anode on opening a constant current, for the non-excitability by slowly rising currents, and for various electrotonic phenomena. A formula is derived for the velocity of nervous conduction, which is verified by experimental data.

5. The generalisation of the above results and their application to the central nervous system opens the way to a physico-mathematical theory of brain activity.

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POSITION EFFECTS ON GENES

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I. INTRODUCTION

ONE of the most fundamental assumptions of genetics is that of the discontinuity of hereditary materials. The germ plasm, transmitted from parents to offspring through the sex cells, is conceived as the sum total of discrete corpuscles, the genes. This conception is, in fact, older than genetics itself, for it was quite clearly envisaged and formulated in the nineteenth century. Despite the widely different phraseology, the atomistic notions of Darwin, Naegeli, and Weismann are profoundly similar to each other, as well as to those of our day.

The theory of the gene is based on the phenomena of Mendelian inheritance. To account for the segregation and the purity of the gametes, genes are supposed to be impermeable to the influences of other genes, even their allelomorphs. The second law of Mendel requires the assumption of at least a relative independence of one gene from the others. The proposition that genes are localized in chromosomes was asserted by Sutton as early as 1903; then definite genes were shown to be located in definite chromosomes (Morgan, 1911; Bridges, 1916). The hypothesis of the linear arrangement of genes in the chromosomes was developed by Sturtevant (1913), resulting in the construction of the genetic chromosome maps showing both the order and the relative distances between the genes in a chromosome. This hypothesis received its vindication in the construction of the cytological chromosome maps (Muller & Painter, 1929; Dobzhansky, 1929 *a, b*).

The gene theory appeared largely completed as far as gene transmission, although not gene action, is concerned. The hereditary material is conceived as absolutely discontinuous. The genes of which it is composed are not interdependent parts of a whole, but rigid, isolated particles. The chromosomes are, then, merely fortuitous agglomerations of genes arranged fortuitously in a linear order. The linear seriation is preserved intact from generation to generation, but it may be occasionally altered, without, however, changing the properties of the resulting organism.

It seems that the above conception of the nature of the germ plasm may prove to be somewhat too crude, so that a rather far-reaching revision may become necessary. The reason for this is the rapidly growing amount of evidence concerning the phenomenon of the "position effect" on genes. It appears that the behavior of a gene is determined not only by its own intrinsic properties, but by the properties of its neighbors as well. An attempt to summarize the available experimental evidence bearing on the problem is presented below. This evidence has been obtained almost entirely by observations on a single object—*Drosophila melanogaster*

II. LETHAL EFFECTS OF TRANSLOCATIONS AND INVERSIONS

The constancy of the linear arrangement of genes in each chromosome of each species may now be considered amply demonstrated. This stability is, however, not inconsistent with the occurrence of changes leading to the emergence of new gene alignments, which are inherited with the same constancy as the original or "normal" alignment. A section of the gene string may be lost (deficiency) or reduplicated (duplication). It may also rotate by 180° remaining in the same place in the chromosome (inversion), or it may be transferred and reattached to a different place in the same or in a different chromosome (translocation). From the point of view of the effects of these different chromosomal aberrations on the organism, an important difference between the deficiencies and duplications on the one hand and the inversions and translocations on the other is apparent. In the former certain genes are either absent, or, *vice versa*, present in more than normal numbers. Hence, individuals carrying deficiencies and duplications may be expected to show, and as a rule do show, various deviations from the ancestral types in their structures and physiologies. Translocations and inversions produce only changes in the relative positions of genes in respect to each other, leaving the number and kind of genes unaffected. Hence, individuals heterozygous, as well as homozygous, for translocations and inversions are expected to be perfectly normal in phenotype. It is a very remarkable fact that this expectation is relatively infrequently realized in practice.

The first translocation discovered in *Drosophila* (Bridges, 1923; Bridges & Morgan, 1923) produces in heterozygous condition a modification of the eye color, and is lethal when homozygous. This translocation involves a transfer of a section of the second chromosome into the third chromosome. The transposed section includes, among others, the wild-type allelomorph of the gene plexus. When located

in a normal, unbroken chromosome, the wild-type allelomorph of this gene is known to be dominant over two doses of the recessive plexus. In the translocation this dominance is appreciably weakened. Two other translocations that arose in *Drosophila* spontaneously (that is, were not induced by X-rays) proved also to produce unexpected effects. One of them (Burkart, 1931) is associated with a "mutation" to a light body color, and the other (Dobzhansky & Sturtevant, 1931) is lethal when homozygous. Since spontaneous mutations are very rare, the association between the chromosome breakages in these translocations and the "mutations" cannot possibly be regarded as a mere coincidence.

Muller's discovery of the production of chromosome rearrangements by X-rays opened wide possibilities for investigations in this field. It soon became apparent that in *Drosophila* only a minority of the induced translocations and inversions are normal in homozygous condition, and the majority are either lethal, or weak, or sterile, or affected somatically (Muller, 1928; Muller & Altenburg, 1930; Dobzhansky, 1929*b*, 1931). In the heterozygotes the abnormalities are met much less frequently than among the homozygotes, but here it should be kept in mind that the technique of the detection of these chromosomal aberrations would by its nature eliminate those amongst them which cause any appreciable decrease in vigor or viability.¹ More important still, the genetic analysis of the abnormalities associated with the translocations and inversions has proved that in most cases they are due to some disturbances in the genes located in the immediate vicinity of the points at which the chromosomes were broken or reattached. A breakage of a chromosome tends to modify the genes adjacent to it.

Patterson, Stone, Bedichek & Suche (1934) have studied on a large scale the viability of homozygous translocations, and the fertility of those among them that proved viable. Their data are summarized in Table I.

Table I. *Viability and fertility of homozygous translocations (after Patterson)*

Chromosomes involved	No. tested for viability	% viable	No. tested for fertility	% fertile
I and II	57	52.6	23	91.3
I and III	71	42.2	30	90.0
I and IV	14	100.0	13	100.0
II and III	120	15.8	19	100.0
II and IV	33	69.6	17	88.2
III and IV	37	48.6	18	88.8

Evidently, only a minority of translocations are completely normal when homozygous. Any analysis of this phenomenon must, of course, take into consideration the possibility of the lethal effects and the sterility of the homozygous translocations being due to chance associations between mutations and breakages

¹ None of the translocations described so far in *Zea mays* differ from the norm either in heterozygous or in homozygous condition (Brink, 1932, 1935). Whether this difference between *Zea mays* and *Drosophila* is significant is uncertain. As compared with those in animals, the translocations in plants may be expected to represent a selected group, since any chromosomal aberration having a lethal or sublethal effect will tend to be eliminated in the haploid generation.

in the same chromosome. In the case of the X-ray induced chromosomal aberrations this possibility is to be examined especially carefully, since mutations affecting the viability are frequently produced by X-ray treatments. Therefore, the problem must be formulated thus: is the association between chromosomal aberrations and the genic changes due merely to chance occurrence of breakages and mutations in the same chromosome, or do these phenomena stand in a causal relation to each other? The frequent coincidence of the loci of mutations with those of chromosome breakages is evidently a strong argument in favor of the second alternative, but quantitative data are needed for a critical evaluation of the situation. Such data were secured by Oliver (1932), who has determined the frequencies of lethal mutations induced by X-ray treatments in the unbroken *X*-chromosomes, as well as in those *X*-chromosomes that became involved in chromosomal aberrations. These frequencies proved to be in one experiment 15.47 ± 2.66 and 31.3 ± 7.82 per cent. respectively, and in another 3.77 ± 0.65 and 23.0 ± 7.89 per cent. respectively. Although Oliver's data are far less extensive than those of Patterson and his collaborators, they justify the conclusion that realignments of chromosomal materials tend to be accompanied by mutations.

Various hypotheses were suggested to explain this fact. Bridges (in Morgan, Bridges & Sturtevant, 1925) supposed that in his translocation (see above) the breakage of the second chromosome was accompanied by a loss of genes adjacent to the point of fracture. Hence, the translocation individuals carry a net deficiency for some genes, with the consequent disturbance of the genic balance and the appearance of phenotypical effects. Sturtevant (1925) discussed the behavior of the gene plexus in the same translocation, and suggested that the functioning of genes may be altered by changes in their relative positions with respect to other genes (position effect). Muller & Altenburg (1930) advanced three possible explanations: (1) The occurrence of one genetic change (breakage or mutation) in a chromosome tends for some unknown reason to be associated with another genetic change in the same chromosome. Such a hypothetical tendency may be called the "group effect". (2) "The alteration in intermolecular surroundings of the genes directly adjacent to the points of breakage and reattachment, in other words the alteration in intergenic contiguities, has in itself brought about a change in the quantity or quality of the physico-chemical action of these genes upon the protoplasm, so as to make them, in effect, somewhat different genes, as though gene-mutations had taken place in the genes on either side of the breakage and attachment points." This is another formulation of the position effect hypothesis. (3) An injury, for instance a destruction, of genes lying in the vicinity of the breakage points in the chromosome.

The experimental discrimination between these different possibilities is admittedly an extremely difficult task. Muller & Altenburg (1930) favored the third of the explanations advanced by them, but subsequently Muller (1932) has turned to the group effect, and still more recently (Muller & Prokofyeva, 1934, 1935; Muller, Prokofyeva & Raffel, 1935) to the position effect hypothesis, which he now considers adequately proven. The position effect hypothesis has been

adopted also by Dobzhansky (1932*a*), Dobzhansky & Sturtevant (1932), Brink (1932), Sivertzev-Dobzhansky & Dobzhansky (1933), Dubinin & Sidorov (1934*a, b*), Dubinin (1935), Schultz & Dobzhansky (1934), Offermann (1935), and some others. The most critical evidence for or against the position effect hypothesis is derived from studies on mutations of previously known loci arising in conjunction with chromosome rearrangements, and especially on repeated origins of similar mutations correlated with breakages of chromosomes in the same general regions. Experiments are so planned as to permit the detection of mutations in a particular locus, and then the presence or absence of chromosome breakages in the vicinity of that locus is determined. *Vice versa*, known chromosomal aberrations in which a given chromosome suffered a fracture in a given region may be tested for mutational changes in genes lying in this region. It should, nevertheless, be kept in mind that the position effect hypothesis was not invoked *ad hoc* for the explanation of the genetic changes observed in chromosomal aberrations, but was arrived at by Sturtevant (1925) on the basis of his studies on the unequal crossing-over at the Bar locus in *Drosophila melanogaster*. It may be well, then, to consider the Bar case first.

III. UNEQUAL CROSSING-OVER AND THE POSITION EFFECT AT THE BAR LOCUS

Among the hundreds of loci in *Drosophila melanogaster* that are known to have produced mutations, the Bar locus is one of the most stable, and yet one of the most unstable ones. Tice (1914) found a single male possessing narrow, band-like eyes; this male proved to carry a dominant sex-linked mutation known as Bar. No further mutations from the wild-type to Bar have been observed since 1914 (except baroid, see below), despite the difference between the Bar and normal eyes being so striking as not to be easily overlooked. In contradistinction to the rarity of the mutations from the wild-type to Bar, the Bar gene itself is highly mutable.

May (1917) reported eleven reversions from Bar to wild-type, and Zeleny (1920, 1921) found that the said reversions occur regularly, with the frequency about 1 : 1600, in strains homozygous for Bar. Moreover, homozygous Bar flies produce (1 : 10,000) still another type, called double-Bar (or ultra-Bar), marked by still narrower eyes than in Bar. In turn, double-Bar reverts to Bar (1 : 2800), or directly to wild-type (1 : 1700). Sturtevant & Morgan (1923) noticed that changes at the Bar locus are always accompanied by crossing-over in the immediate vicinity of that locus in the chromosome; the nature of this correlation was analysed by Sturtevant (1925, 1928). The demonstration of the dependence of the mutations at Bar upon crossing-over is technically simple. For instance, females are obtained that are homozygous for Bar, but heterozygous for the two genes, forked and fused, lying in the chromosome to the right and to the left of Bar respectively (Fig. 1). Any double-Bar or wild-type fly found in the offspring of such females is a cross-over between forked and fused, although not all the cross-overs are "mutations" at the Bar locus. Since the frequency of crossing-over between forked and fused is 2.7 per cent., and the frequencies of "mutations" at the Bar locus are less than

0.1 per cent., the relation observed is evidently not accidental. Since crossing-over takes place in *Drosophila* in females but not in males, changes at the Bar locus (as noticed already by Zeleny) do likewise.

According to Sturtevant, the apparent mutations at the Bar locus are due to

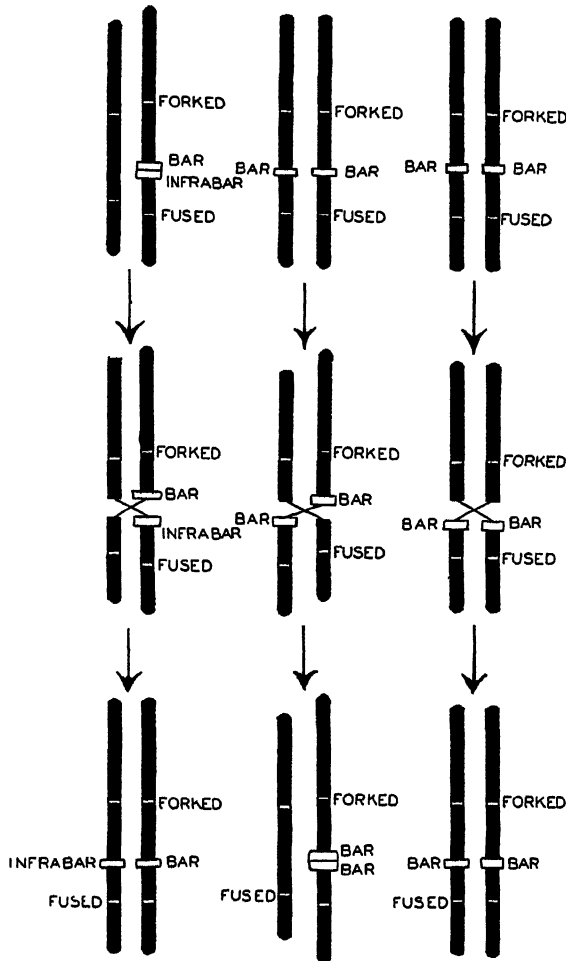


Fig. 1. Equal (right column) and unequal (middle and left columns) crossing-over at the Bar locus in the X-chromosome of *Drosophila melanogaster*.

unequal crossing-over. Normally crossing-over is equal: the homologous chromosomes are broken at precisely the same levels, between the loci of the same two genes, so that the exchange of fragments results neither in an addition nor in a loss of a single gene to either chromosome (the right column in Fig. 1). But chromosomes carrying Bar may be broken—one to the right and the other to the left of the Bar locus. The exchange of segments must result, consequently, in formation of one chromosome bearing two Bar genes and of one chromosome bearing none (the

middle column in Fig. 1). The chromosome with two Bar loci gives rise to the "mutation" double-Bar, and the chromosome without Bar to the reversion to wild-type. It follows that the "mutations" at the Bar locus are not mutations in the usual meaning of the term (not alterations of the structure of the gene itself), but merely reduplications and losses of a gene.

The more orthodox kind of mutations may also take place at the Bar locus. Sturtevant (1925) found in the progeny of a Bar male a single individual with eyes intermediate in size between Bar and wild-type. The new type was called infrabar. Since infrabar arose in the gametogenesis of a male, its origin cannot be due to unequal crossing-over, but rather to an alteration of the structure of the Bar locus itself. The behavior of infrabar is analogous to that of Bar; by unequal crossing-over it reverts to the wild-type, and gives rise to a more extreme type, known as double infrabar. Double-infrabar reverts to infrabar and to wild-type, but never to Bar. More important still, from females heterozygous for Bar and infrabar Sturtevant obtained by unequal crossing-over two kinds of individuals combining Bar and infrabar in the same chromosome. In some of these the gene order was found to be forked—Bar—infrabar—fused, and in others forked—infrabar—Bar—fused. These two gene orders are retained in the offspring of a given fly just as tenaciously as any gene order in a normal chromosome (the left column in Fig. 1). This experiment affords a crucial test of the unequal crossing-over hypothesis. Its validity has been proved also by an independent method by L. V. Morgan (1931, cf. Thompson, 1931).

Thus, it may be taken as established that double-Bar is not a single locus at all, but two Bar genes lying side by side in the same chromosome, potentially separable from each other by crossing-over. Similarly, two infrabar genes give rise to double-infrabar, and the Bar-infrabar is composed of one Bar and one infrabar gene lying side by side. Since all the known allelomorphs of Bar produce a decrease of the eye size, their phenotypic effects may be measured quantitatively in terms of the facet numbers. Sturtevant (1925) studied the number of facets in individuals carrying all the possible combinations of the Bar allelomorphs. Some of the results obtained by him were most unexpected. A female carrying one wild-type X-chromosome and one X-chromosome with double-Bar (heterozygous double-Bar) has two Bar genes, and her eyes contain 45.42 ± 0.24 facets each. A female homozygous for Bar has also two Bar genes, and hence should have eyes similar to those of the heterozygous double-Bar females. In reality, homozygous Bar females have 68.12 ± 1.09 facets. Similarly, a female heterozygous for double-infrabar and a female homozygous for infrabar carry each two infrabar genes, and hence should have eyes of identical size. This is again not the case: the former females have smaller eyes (200.2 ± 8.6 facets) than the latter (348.4 ± 12.4). Females of the genetic structures Bar-infrabar/wild-type and Bar/infrabar should for the same reason be similar, but the former have 50.46 ± 0.40 and the latter 73.53 ± 1.29 facets.

The discrepancies observed reveal an interesting fact: two Bar allelomorphs located in the same chromosome (two Bar genes in double-Bar, two infrabars in double-infrabar, Bar and infrabar in Bar-infrabar) produce a stronger effect on the

eye size than the same two genes would produce if located in separate chromosomes. Bar allelomorphs reinforce each other's action if they lie side by side in the same chromosome. The action of the Bar gene is influenced by its neighbors; this is the phenomenon of position effect.

IV. THE CASE OF BAROID

Sturtevant's work has elucidated the mechanism of the "mutations" at the Bar locus, leaving the problem of the original mutation from wild-type to Bar rather in the dark. The wild-type carries no Bar locus at all, it is a no-Bar. It follows that the origin of Bar from wild-type is either a creation of a new gene, or else the Bar locus has been brought to its present location in the chromosome from elsewhere. The first of these alternatives being rather difficult to conceive, Wright (1929) suggested that Bar is a duplicating fragment of some chromosome attached to the *X*-chromosome. Essentially the same notion, expressed in very different phraseology, has been advanced by Thompson (1931).

The appearance of a new mutation from the wild-type to an allelomorph of Bar, called baroid, permitted further investigation of the problem (Dobzhansky, 1932 *a*). Baroid arose in a single daughter of a wild-type male treated with X-rays. It produces the same kind of external effects as the previously known Bar allelomorphs—a reduction of the eye size. In heterozygotes with Bar baroid intensifies the action of the former. The locus of baroid in the *X*-chromosome is, as far as possible to ascertain, identical with that of Bar. In short, from the standpoint of every known criterion of allelomorphism baroid behaves as a new allelomorph of Bar.

A genetic study of baroid disclosed a relation between the origin of this mutation and that of a chromosomal aberration with which it is associated. The *X*-chromosome carrying baroid was broken at that locus, while the second chromosome underwent a fracture not far from the middle of its right limb. The fragments were exchanged, so that the chromosomes of the baroid strain have the structure indicated in Fig. 2; it is a reciprocal translocation involving the *X* and the second chromosomes. The origin of baroid not only coincided in time with that of the translocation, but the locus of baroid is adjacent to the break in the *X*-chromosome. (It is not possible to determine whether baroid lies together with fused in the chromosome retaining the *X* spindle fiber, as indicated in Fig. 2, or in the fragment carrying forked and now attached to the second chromosome; however that may be, baroid is not separable from the break by crossing-over.) The natural presumption is that the origin of the baroid "mutation" is causally related to the chromosome fracture.

The seemingly simplest supposition as to the nature of this relation is that some gene or genes were destroyed at the point of the chromosome fracture, and the deficiency thus obtained produced the effects of baroid. This supposition leads, however, to a difficulty: according to Sturtevant wild-type flies have no Bar locus, hence the origin of baroid can hardly be a loss. On the other hand, the position

effect hypothesis permits a satisfactory account of the situation. A gene normally located in the *X*-chromosome of *Drosophila melanogaster* in the region of the loci forked and fused (Fig. 2) causes the development of normal eyes. If, however, the association between this gene and its normal neighbors is disrupted, or else it

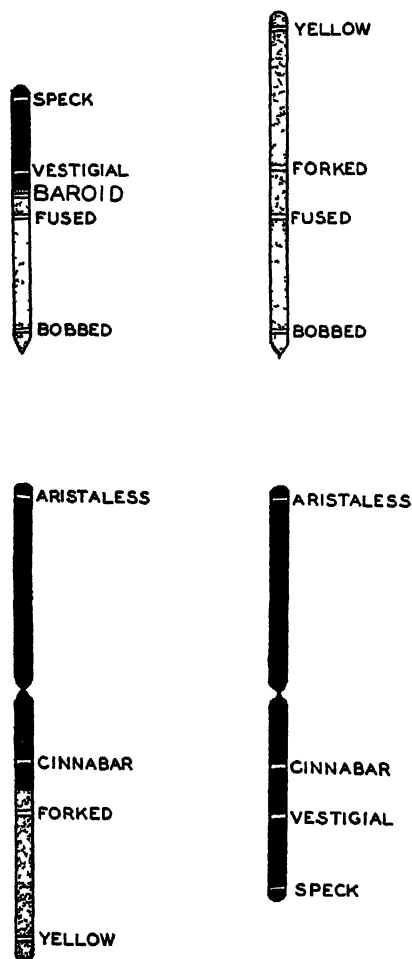


Fig. 2.

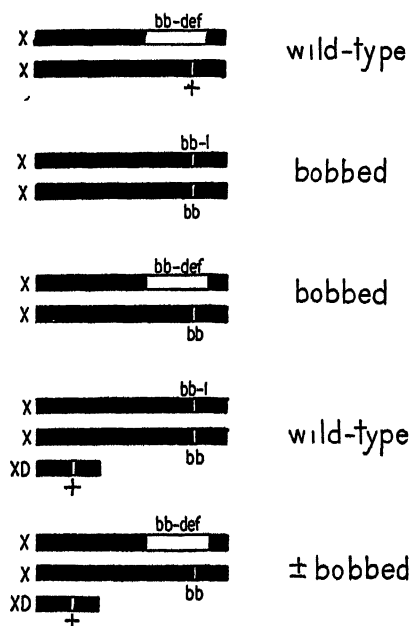


Fig. 3.

Fig. 2. The structure of the *X* and the second chromosomes in the baroid translocation. The *X*-chromosome—stippled; the second chromosome—black. Normal chromosomes right, translocation chromosomes left.

Fig. 3. Dominance of the wild-type allelomorph of bobbed over its recessive allelomorphs (after Sivertzev-Dobzhansky & Dobzhansky, 1933).

becomes associated with genes with which it is but normally in contact, it may so change its behavior as to produce Bar eyes. Indeed, in baroid a substitution of genes normally located in the second chromosome for those normally lying in the *X* in the forked-fused region has taken place. In the case of the mutation from wild-type

to Bar one can, following Wright, assume that a very small fragment of some chromosome has become inserted into the *X*-chromosome, and that an interaction between a gene, or genes, in that fragment and the *X*-chromosome genes is responsible for the origin of Bar (Dobzhansky, 1932*a*). (Note added in proof: Dr C. B. Bridges kindly informs me that the *X*-chromosomes carrying double-Bar, Bar, and no-Bar are cytologically different from each other. In Bar, with the aid of the salivary gland chromosome method, three bands, two of which may be doubles, may be seen in the part of the chromosome between the loci of forked and fused. These bands are lacking in the chromosome of wild-type flies derived from Bar by reversion or unrelated to Bar. In double-Bar six extra bands may be seen in the same region of the chromosome; these six bands form a small "repeat region" consisting of two similar sections of three bands each. This discovery affords a conclusive proof of the above interpretation of the nature of the Bar "gene").

Offermann (1935) observed a translocation in which an *X*-chromosome carrying Bar and the fourth chromosome are involved. The break in the *X*-chromosome took place close to the Bar locus; Offermann states that this has led to an intensification of the Bar effects on the eye size. Similar phenomena have been observed also by the writer (unpublished) in at least two duplicating fragments in which the *X*-chromosome carrying Bar has been broken close to, but not immediately at, the Bar locus. It seems that the Bar locus is very sensitive to position effects, but whether or not every chromosome break in its vicinity leads to visible alterations of its functions remains to be studied.

V. CHANGES AT THE LOCUS OF BOBBED

Bobbed is the only gene known to have allelomorphs both in the *X* and in the *Y*-chromosomes of *Drosophila melanogaster*. In the *X*-chromosome bobbed is located at the extreme right end of the genetic chromosome map; in cytological terms it lies close to the spindle fiber attachment, well in the "inert region" of the chromosome. In the *Y*-chromosome bobbed is also not far from the spindle attachment (Stern, 1929). Mutations from the wild-type to bobbed are relatively frequent; the mutant allelomorphs produce a series of phenotypic changes among which a reduction of the bristle size is the most striking.

Stern (1929) and Stern & Ogura (1931) described several translocations in which sections of the *Y*-chromosome were attached to the *X*-chromosome. In some cases mutations to allelomorphs of bobbed arose simultaneously with the translocations. The frequency of these cases seems too high to be a coincidence. A mutation at bobbed taking place concomitantly with a translocation from the *X*-chromosome to the third chromosome was described also by Sidorov (1931*b*).

The *X*-chromosome may be fragmented by X-rays; some fragments are lost while others are recovered in the offspring of the treated fly as duplications. In a majority of cases the recovered fragments consist of sections of the distal end of the chromosome united with parts of its proximal end (the latter including the spindle

attachment), the middle portion of the original chromosome being lost. If the duplicating fragments are obtained in the offspring of irradiated wild-type flies, the fragments may either contain the wild-type allelomorph of bobbed or not contain the bobbed locus at all (depending upon whether the bobbed locus is included in the lost or in the recovered portion of the original *X*-chromosome). The fragments cannot contain mutant allelomorphs of bobbed, unless mutations take place at the time of irradiation. Whether or not a given duplicating fragment includes the locus of bobbed can be determined by a simple test: females or males are obtained which carry the recessive allelomorphs of bobbed in their *X*- and *Y*-chromosomes, and which carry the fragment in question (Fig. 3). If the bobbed locus is not included in the fragment, the flies must manifest the effects of bobbed; in the opposite case the flies must be wild-type in phenotype. (The wild-type allelomorph of bobbed is apparently completely dominant over its recessive allelomorphs.)

While studying some fragments by the method just outlined, Sivertzev-Dobzhansky & Dobzhansky (1933) were confronted by an unexpected situation. Five out of the six duplications studied behaved as though they contained allelomorphs of bobbed of various strength, intermediate between wild-type and the recessive bobbed, and one duplication appeared to be deficient for bobbed. The five duplications proved to be effective enough to suppress the manifestation of weaker bobbed allelomorphs (bobbed, bobbed-lethal), but not of the strongest one (bobbed-deficiency). The normal wild-type allelomorph of bobbed suppresses the effects of bobbed-deficiency entirely (Fig. 3). Hence, the dominance of the wild-type allelomorph is reduced if it lies in a chromosome fragment instead of a normal *X*-chromosome.

Among the six duplications studied, none retained a normally functioning wild-type allelomorph of bobbed. In all of these duplications the chromosome has been broken in the vicinity of the bobbed locus. It follows either that mutations in that locus were induced together with every breakage, or else that the behavior of bobbed is due to position effects. Some other possible, although not probable, explanations of the same facts were discussed by Sivertzev-Dobzhansky & Dobzhansky (1933); they need not be considered here.

VI. THE DOMINANCE OF CUBITUS INTERRUPTUS IN TRANSLOCATIONS

The gene *cubitus interruptus* is located in the very minute fourth chromosome of *Drosophila melanogaster*. Its mutant allelomorph causes a reduction of the wing veins, this effect being completely recessive to that of the wild-type allelomorph. Dubinin & Sidorov (1934*a, b*; Dubinin, 1935) investigated a number of translocations involving the fourth chromosome and various others (the third, second, *X*, or the *Y*-chromosome). All these translocations were obtained in the progeny of wild-type males treated with X-rays, and hence, barring possible mutations, must carry the wild-type allelomorph of *cubitus interruptus*. Nevertheless, when individuals heterozygous for the translocations were made heterozygous also for

the recessive cubitus interruptus, the effects of the latter manifested themselves in some instances (Fig. 4). More precisely, out of the thirty-eight translocations tested, eighteen failed to suppress cubitus interruptus, and twenty showed a complete dominance (Dubinin, 1935). Following Dubinin & Sidorov, Sturtevant and the writer tested ten other translocations involving the fourth chromosome (one *X-IV*, four *II-IV*, and five *III-IV*); four among these produced a more or less incomplete, and six a complete suppression of cubitus interruptus. The count stands now: twenty-six translocations behaving normally, and twenty-two permitting the recessive cubitus interruptus to manifest itself in various degrees. What properties of a given translocation determine its behavior with respect to cubitus interruptus is unknown; a comparative cytological investigation of the translocations with the aid of the salivary gland chromosome method might elucidate the situation.

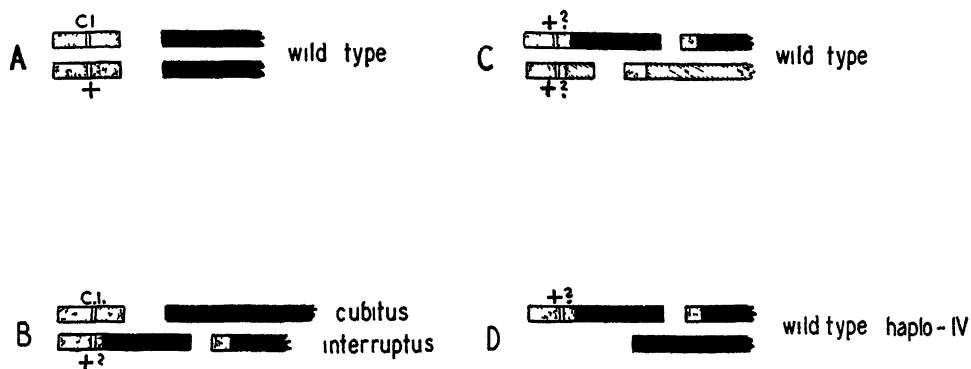


Fig. 4. Dominance of the wild-type allelomorph of cubitus interruptus lying in a normal fourth chromosome (A), and in a fourth chromosome involved in translocations (B, C, D). *C.I.* the recessive cubitus interruptus; + its wild-type allelomorph lying in an unbroken chromosome; + ? its wild-type allelomorph lying in a translocation chromosome.

The behavior of cubitus interruptus in translocations is clearly analogous to that of bobbed in duplicating fragments. Dubinin & Sidorov were able, however, to carry the analysis much further than was possible in the bobbed case. The facts suggest that the wild-type allelomorph of cubitus interruptus may be modified by position effects. An alternative explanation is that mutations take place in this gene simultaneously with the induction of translocations involving the fourth chromosome. If the latter premise is granted, the translocations failing to suppress cubitus interruptus carry in their fourth chromosomes mutated allelomorphs, or even deficiencies, of this gene. Consequently, a manifestation of cubitus interruptus characteristics may be expected in individuals homozygous for the translocations, as well as in individuals heterozygous for two different translocations each of which fails to suppress cubitus interruptus (Fig. 4 C). According to Dubinin & Sidorov, this expectation is not realized. A still more rigorous test is provided by studies on flies carrying the translocations and lacking the free fourth chromosome (in other words deficient for one fourth chromosome, the Haplo-IV condition, Fig. 4 D).

Such flies carry a single allelomorph of cubitus interruptus, namely that lying in the fourth chromosome involved in the translocation. Deficiencies are known to produce an exaggeration of the effects of recessives whose loci are included in the deficient section of the chromosome. This relation is very clear in eyeless Haplo-IV flies. Hence, were the cubitus interruptus locus in the translocations mutated, the characteristics of the mutation would have the best chance to manifest themselves in Haplo-IV flies. Dubinin & Sidorov found no trace of cubitus interruptus in such flies. The conclusion is to be drawn either that the behavior of cubitus interruptus is due to position effects involving a decrease in dominance of the wild-type allelomorph, or that mutations in hypothetical genes that are specific modifiers of cubitus interruptus but produce no effect of their own are induced in translocations.

One of the translocations described by Dubinin & Sidorov (1934*a, b*) involves an exchange of fragments between the fourth and the third chromosomes; the third chromosome is broken somewhat to the right of the locus of the gene hairy. In spite of the fact that this translocation was obtained in the offspring of a wild-type fly, the individuals heterozygous for it and for the recessives cubitus interruptus and hairy manifest the effects of both these recessives. The dominance of the wild-type allelomorph of hairy has been, consequently, impaired, presumably due to a position effect. By studying crossing-over between an unbroken third chromosome carrying the wild-type allelomorph of hairy, and the third chromosome involved in the translocation, it has been found possible to replace the "weakened" gene in the broken chromosome by the gene from the normal chromosome. In his most recent paper Dubinin (1935, p. 356) states that such a replacement leads to a "weakening" of the gene thus introduced into the broken chromosome. Here, I believe, a crucial proof of the position effect hypothesis is afforded; the publication of the details of this remarkable experiment is awaited with great interest.

VII. CHANGE IN THE DOMINANCE OF GENES LYING IN DUPLICATING FRAGMENTS

In *Drosophila* the dominant allelomorphs are as a rule strong enough to produce a more or less complete suppression of the effects of one, or even of two, doses of the corresponding recessives. Provided the dominance of a gene is solely a property of that gene itself, it should make no difference whether it lies in a normal or in a broken chromosome. As shown above, the behavior of some genes seems incompatible with this proviso. The dominant allelomorphs of plexus, bobbed, cubitus interruptus, and hairy appear "weakened" if the chromosome carrying them undergoes fragmentation close to their loci.

Dobzhansky & Sturtevant (1932) have published a preliminary account of several duplications containing the loci of the second chromosome genes purple and light, and the sex-linked genes yellow, kurz, rudimentary, and forked. In all cases the duplications arose by fragmentation of normal chromosomes carrying the wild-type allelomorphs of the genes in question. Nevertheless, individuals carrying these duplications in addition to normal chromosomes with the respective recessive

genes are not wild-type in phenotype. The suppression of the effects of the recessives is more or less incomplete. The weakening of the wild-type allelomorph of yellow has been observed in five duplications, of forked in three, and of rudimentary in two. All the duplications showing a modification of the effects of a certain gene have some properties in common, namely, in all of them the chromosome has been broken in a certain region, not far from, but not necessarily adjacent to, the loci of the genes affected. Conversely, the duplications not showing the modification of the same genes also have common properties: in these the chromosome is usually fragmented at levels more distant from these genes. In general, the closer the locus of a gene lies to the point of the chromosome fracture the more likely is the gene to be modified, albeit the modification may extend for a certain distance from the fracture.

The assumption that the dominance of a gene is a function not only of its own structure but also of the structure of its neighbors leads to no difficulties in explaining the above facts. It must be granted, however, that as a proof of the position effect hypothesis the evidence derived from the study of the duplication fragments is relatively least convincing. Individuals carrying duplications have an altered genic balance; this alteration may be responsible for the changes in the dominance relationships. Against this possibility argues the observation that the weakening of dominance is observed only in the genes included in the duplicating fragments, and not in the genes lying elsewhere in the chromosomes. In consequence of this, the genic balance explanation may be retained only at the price of resorting to an accessory hypothesis, namely, that the dominance of a gene is determined not by the genic balance in general, but by the dosages of the genes lying in the chromosome in the immediate vicinity of the gene in question. Although there is no independent evidence in its favor, this possibility cannot be dismissed too lightly. Mutations in the genes lying in the duplications afford a second alternative explanation. This possibility will be considered in more detail below.

VIII. MUTATIONS IN THE LEFT END OF THE X-CHROMOSOME

The left (distal) end of the *X*-chromosome is probably the most extensively studied region in the chromosomes of *Drosophila melanogaster*. Some of the genes located in this region are among the most frequently mutating ones in the species (white, scute, yellow). The fact which is especially interesting for us now is that some chromosomal aberrations, both inversions and translocations, having breakages in this part of the *X*-chromosome, arose concomitantly with mutational changes affecting the genes in the same region. The spontaneous translocation of Burkart (1931) and Burkart & Stern (1933) has already been mentioned. The individuals carrying the translocation differ from normal in some characteristics, among which a change in the bristle color is most obvious. The *X*-chromosome is broken in this translocation not far from the left end; the phenotypical effect depends upon a locus adjacent to that of the chromosome fracture.

After the discovery by Muller of the X-ray technique of the production of

mutations and chromosomal aberrations, many new changes in the left end of the X-chromosome were observed, especially mutations of the gene *scute*. Dubinin (1929*a, b*) and Serebrovsky & Dubinin (1930) described the allelomorph *scute-2* connected with a translocation. Agol (1931, 1932) studied *scute-4* connected with an inversion. Similar relations were observed also in some other *scute* allelomorphs (Shapiro, 1930; Levit, 1930; Sidorov, 1931*a*; Agol, 1932; and others). Among about thirty *scute* allelomorphs known at present a large part is associated with chromosome breakages in the general neighborhood of the *scute* locus. An allelomorph of yellow associated with an extensive inversion is also known (Serebrovsky *et al.* 1928; Dubinin & Friesen, 1932). From the standpoint of the possible application of the position effect hypothesis these cases were until recently rather unsatisfactory, for the chromosomal aberrations involved in them were not described in detail (which is, of course, not surprising since their finders were interested in using them for different purposes). The situation is now changed, since the problem has been subjected to a searching analysis by Muller.

Muller (1932) discovered that although some mutations that have been found together with chromosomal aberrations arose at loci immediately adjacent to chromosomal breakages, others appeared at a small but measurable distance therefrom. Thus, a *scute* allelomorph was found in a broken chromosome (*X-24*), but the breakage took place not at the *scute* locus but definitely to the right of it. An eversporting (see below) allelomorph of white arose in a chromosome that was broken to the right of the white locus. Indeed, in some *scute* allelomorphs the mutation took place, as far as possible to determine, immediately adjacent to the locus of the chromosome fracture (League's *scute-19*, Muller, 1932; *scute-8*, Patterson, 1932, 1933; Patterson & Stone, 1935, *scute-4*, *scute-7*, *scute-8*; Beadle & Sturtevant, *in press*). And yet the fact that some mutations appear at a distance from the break requires a careful examination, Muller (1932) undertook experiments especially designed to clear up this point, and discovered that sometimes groups of mutations appear simultaneously at closely neighboring, though separate, loci, with or without association with chromosomal rearrangements (*scute-J 1*, a *scute* and an ommatidial effect in *achaete-3*, a scarlet allelomorph and a lethal mutation at a locus close to scarlet).

These facts were taken by Muller (1932) as indicating that there is a causally conditioned tendency for one gene mutation to be accompanied by another nearby and for one genetic change in the chromosome (*e.g.* a breakage) by another change (a breakage or a mutation) at a close distance. This is the group effect hypothesis, already advanced by Muller & Altenburg (1930), as a possible explanation of the correlation between mutations and chromosome breakages. Muller (1932) wrote: "I do not wish to deny the possibility of a position effect.... I believe, however, that the third alternative, that of a real gene mutation, is more probable as an explanation of the phenomenon here in question, especially in consideration of related phenomena." In the opinion of the present writer, the existence of group effects is still an open question, but in any case an occurrence of a mutation at a small distance from a breakage point does not necessarily preclude the possibility

of this "mutational" change being due to a position effect. Indeed, we know as yet too little about the possible mechanisms of position effects to maintain that the influence of a breakage cannot extend beyond the genes immediately adjacent to it. The only condition under which such an opinion may be justified is if the induced mutation can be separated by crossing-over from the breakage without losing its properties.

In any event, the facts advanced by Muller in 1932 appear now in a very different light, due to the further explorations of Muller himself. By the analysis of the giant salivary gland chromosomes, Muller & Prokofyeva (1934, 1935) have found that the chromosome breakages associated with the mutations yellow-3 P, yellow-4, scute-8, scute-19, and scute-L8 have taken place in the immediate vicinity of the respective genes. More important still, in some of the cases where a mutation seemed to have been induced at a distance from the chromosome fracture, or where two separate mutations were induced in conjunction with a single break, Muller, Prokofyeva & Raffel (1935) now find very minute rearrangements of the inversion type that were overlooked by the cruder old methods. In such a way, the double mutations lethal-J1 and scute-J1, achaete-3 and the ommatidial disarrangement now are shown to lie each at a separate break. Similar chromosomal rearrangements are associated with the appearance of similar mutations (scute-4 and scute-L8). This fact Muller & Prokofyeva (1935, p. 22) consider "crucial evidence from another direction for the position effect hypothesis". It should be pointed out that evidence of the same nature has been obtained also in other cases (duplications, mutable eye colors).

IX. DOMINANT EYE COLORS

A series of mutants producing dominant changes in the eye color were found in *Drosophila melanogaster* by Weinstein (1928), Muller (1930), Van Atta (1932 *a, b*), Glass (1933, 1934), Schultz & Dobzhansky (1934), Dubinin (1934), and others in the offspring of X-ray treated flies. As pointed out first by Muller (1930), all these mutants have some remarkable properties in common. As already mentioned, they are dominant to the wild-type, while all the spontaneous and a majority of the induced eye-color mutants are recessive. Second, they are associated with chromosomal aberrations of various kinds—inversions, translocations, deficiencies, and duplications. The loci of the mutants lie either adjacent to or not very far removed from the chromosome breakages. Third, most of the mutants are "eversporting", or somatically unstable, so that the eyes are usually not evenly colored, but consist of patches of tissues of different colors. The last property, that of the mutability, attracts especial attention; considerable work has been done in this connection without, however, bringing about a satisfactory solution of the riddle. This aspect of the situation is highly complicated, and belongs to a special field, so that it must be left out of our discussion.

A group of the dominant eye colors that is most interesting for us at present are the so-called Plum allelomorphs (Muller, Van Atta, Glass, Schultz & Dobz-

hansky, *loc. cit.*). Plum (or Dilutes, as they are otherwise also called) flies have eyes of a brownish color with splotches of a darker hue. All the known Plum are allelomorphic to each other, and also to the recessive mutant brown. Brown produces, as its name implies, a brown eye color without the differently colored patches. Brown is located in the second chromosome, not far from the right end of the latter. The point of importance is that in all Plum allelomorphs for which exact data are available the second chromosome carries a chromosomal aberration, usually an inversion, involving a breakage in the immediate vicinity of the brown locus. Thus, a fragmentation of the second chromosome in this particular region is at least frequently, and perhaps always, accompanied by a mutation in the brown locus.

In some Plum allelomorphs an inversion is found in which the second chromosome has been broken at brown, and also near to the locus of the gene light. The spontaneous mutations at the light locus are eye-color changes, not of the ever-sporting type, of a somewhat different type than in brown. Light and brown are, of course, not allelomorphic. The locus of light in the second chromosome is normally far removed from that of brown; while brown lies close to the right end of the second chromosome, light is not far from the spindle fibre attachment which in this chromosome is median. Characteristically enough, two Plum allelomorphs associated with inversions of the type just described proved to be allelomorphic not only to brown but also to light. Unfortunately data are not as yet published to show whether or not all Plum with such inversions show this double allelomorphism.

X. CONCLUSIONS

The position effect hypothesis assumes that a gene does not remain the same if it is removed from its normal location in the chromosome and placed in a new location. This is so radical a departure from the conventional concepts of genetics that a most exacting scrutiny of the evidence is advisable before the hypothesis is accepted. One is nevertheless forced to the conclusion that in the case of Bar *v.* double-Bar a demonstration of position effect has been completed. The same seems to be true in the case of the gene hairy; here, however, the pertinent evidence has not yet been fully published. All other alleged cases of position effects may be explained on other assumptions. These other assumptions involve *ad hoc* hypotheses, and, what is especially noteworthy, different ones for almost every case. For example, in baroid one is forced to assume the existence, at least in some strains, of a wild-type allelomorph of Bar, which is contrary to the evidence obtained by Sturtevant. The decrease of the dominance of the genes lying in duplicating fragments is to be explained as due to dominance modifiers concentrated always in the vicinity of the genes modified in the chromosome.

The most elusive of the supplementary hypotheses is that of the group effect, if applied as an alternative to position effect. The former assumes that the occurrence of a breakage in the chromosome increases the likelihood of the appearance of a mutation in the adjacent genes—genetic changes tend for some reason to occur in groups. This is certainly true, but in my opinion a restatement of the facts and not a causal explanation is thus being offered. Most serious are the difficulties of

the group effect hypothesis when it is confronted by the repeated origin of the same "mutation" at the loci of breakages in similar chromosome rearrangements, as in the cases of Plum, scute, and probably also bobbed and cubitus interruptus. Here the evidence tends to show that similar, or even identical, mutations arise *every time* the gene order is altered in a definite fashion. Whether or not these cases as they stand now constitute crucial evidence for the position effect hypothesis (as Muller and Prokofyeva seem to believe), they are certainly best accounted for by it. The difference between the position effect and the group effect hypotheses becomes here a matter of words. This is, of course, not to deny that "group effects" in gene mutations may exist; the question is wide open for experimental attack. The kernel of the problem is whether or not a mutation at a definite locus is regularly associated with definite mutations in one or several other loci, and, if this were the case, whether these multiple mutations can be separated from each other by crossing-over without losing their properties.

The experimental evidence is now too meager for a study of the possible mechanisms underlying position effects. An interesting attempt in this direction has been made by Offermann (1935), to whose paper the reader is referred. Perhaps the main problem in this field is whether or not position effects are reversible. A gene changes its behavior due to a disruption of its associations with the genes lying normally next to it, or due to the formation of new associations. Will this gene recover its normal behavior if the original gene order is restored? In the case of Bar such a reversibility has been actually observed: Bar and infrabar derived from the Bar-infrabar compound are similar to the respective genes that have entered into the combination to form the compound (Sturtevant, 1925). According to Dubinin (1935), a wild-type allelomorph of hairy introduced into the broken chromosome by crossing-over suffers a decrease in dominance; Dubinin does not state whether the dominance is reimpacted to a gene extracted from the broken chromosome into a normal one.

The reversibility of position effects indicates that the causes, whatever their nature, that are instrumental in modifying the properties of the genes affected, are operative not only at the time of the original fracture of the chromosome, but remain so indefinitely. The activities of a gene lying in an abnormal position in the chromosome are deflected from their normal course by influences emanating from its new environment, but no permanent alteration is wrought in the gene itself. As pointed out, among others, by Muller & Prokofyeva (1934, 1935), this may mean that position effects are "due to a higher degree of interaction between locally more concentrated *products* of gene activity than between more distantly produced and either more diluted or changed products". In other words, position effects may prove to be essentially developmental phenomena, but phenomena of intracellular physiology, affecting the co-ordinates on which the reactions between the primary gene products take place.

On the other hand, it is possible to visualize also irreversible position effects. The genes may be pictured as organic molecules united with each other in the longitudinal direction to form micelles, after the fashion of the cellulose micelles.

Breakage of such a micelle, and the formation of a new micelle, may involve modifications of the intergenic bonds which may or may not be reversible in case the original gene order is restored. An irreversible position effect is a change in the gene structure, and therefore a mutation by definition, but a mutation invariably accompanying a certain alteration of the gene order in the chromosome. It is not unlikely that some changes in gene position may induce reversible, and other changes irreversible position effects. In this case two rather diverse phenomena are included at present under the common name of "position effects".

Irrespective of what the mechanisms of position effects are, the existence of these phenomena throws new light on the problem of the structure of the germ plasm. The age-old antinomy between the whole *versus* its parts presents itself in a remarkably concise form. The hereditary material is discontinuous, for it is segregated into independent units, *genes*. And yet, it is a continuum of a higher order, since the independence of the units is incomplete—they are changed if their position in the system is altered. A chromosome is not merely a mechanical aggregation of genes, but a unit of a higher order, since another chromosome containing the same genes differently arranged may be a different chromosome. The properties of a chromosome are determined by those of the genes that are its structural units, and yet a chromosome is a harmonious system, which reflects the history of the organism, and is itself a determining factor of this history.

These considerations are of some importance, especially in view of the growing amount of evidence on the role played by changes in gene alignments in the evolutionary process. That chromosomes of different species may contain different genes, or genes differently arranged, has been long suspected by cytologists. More recently it has become clear that gene rearrangements occur in evolution on a hitherto unsuspected scale. The unpublished observations of Tan and the writer (by the salivary gland chromosome technique) on the gene alignment in two related species, *Drosophila pseudoobscura* and *D. miranda*, show that some sections of chromosomes have the gene order so profoundly modified that the deciphering of the changes that have taken place is possible only with difficulty. Less closely related species (e.g. *D. melanogaster* and *pseudoobscura*) have the gene order modified apparently beyond recognition. To what extent differences between species may be due to position effects remains to be studied.

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ON THE EVOLUTION OF BONY FISHES DURING THE TRIASSIC PERIOD

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I. INTRODUCTION

THE past fifteen years, particularly the latter third of that period, has witnessed remarkable advances in our knowledge of Triassic fishes. Before this period the fishes known from the Triassic did not compare in importance with the imposing collections from the upper Palaeozoic Rocks or with the numerous and varied holosteans and teleosts from the Jurassic and Cretaceous. Indeed, one gained the impression that Triassic fishes were relatively scarce, and their detailed structure was not very well known.

The fossil fish material from the British Trias was rare and often fragmentary. The most extensive collections had been made from the middle and upper Alpine Trias. Varied faunas were known from Perledo, Besano, Hallein, Seefeld, etc. (Allessandri, 1910; Gorgonovic-Kramberger, 1905; Kner, 1866; Deecke, 1889). Fossil fishes, often of a fragmentary nature, were also known from the Continental German Muschelkalk (Stolley, 1920).

Though Triassic fossil fishes had been recorded from South Africa (Woodward, 1893; Broom, 1909), Madagascar (Woodward, 1910), and Spitsbergen (Woodward, 1912), the only extensive faunas known outside of Europe were obtained from the Hawkesbury Series of New South Wales, and a more restricted one from the upper Triassic of Eastern North America.

The Hawkesbury fishes were obtained from two distinct horizons, and were monographed by Smith Woodward (Woodward, 1890, 1908). The North American fauna was distinguished by the fact, that although large numbers of individuals were known, only three genera were certainly represented (Eastman, 1911, 1914).

These Triassic fishes as a whole told us scarcely more than that the great palaeoniscid group had died out almost completely, and by upper Triassic times had been replaced almost entirely by holostean fishes.

The modern era in the study of Triassic fishes may be said to have been inaugurated by Stensiö in 1921, with the publication of the first part of his work on the Triassic fishes of Spitsbergen (Stensiö, 1921). This classical work was completed with the publication of the second part in 1925 (Stensiö, 1925).

In 1929 a fine collection of excellently preserved fishes was made by Prof. D. M. S. Watson from rocks of lower Triassic age in the Karroo series of South Africa. The description of this fauna appeared soon after (Brough, 1931, 1933). This locality had yielded fossil fishes previously, but not to the same extent.

About the same time other important collections were being assembled:

(1) *Madagascar*. Fossil fishes of lower Triassic age had been collected by French geologists and engineers and a quantity of material was accumulated in France. Dr E. I. White of the British Museum also visited Madagascar and obtained an extensive collection of lower Triassic fishes. The Madagascar fauna has been worked in recently by a number of individuals (Brough, 1933; White, 1932; Moy-Thomas, 1935; Piveteau, 1935). Dr Piveteau's publication is a monograph dealing in detail with a number of genera.

(2) *Australia*. The Rev. R. T. Wade collected, and subsequently described, a fish fauna from a new horizon in the Hawkesbury series of New South Wales (Wade, 1932, 1933, 1935).

(3) *Greenland*. The successive Danish expeditions of recent years to East Greenland brought back large numbers of beautifully preserved fossil fishes from the basal beds of the Triassic. This fauna was elaborately described by Prof. Stensiö (Stensiö, 1932).

The new faunas listed above were particularly valuable in that all but one (Hawkesbury beds presumed middle Triassic)¹ are lower Triassic in age. A gap is thus closed, for the Triassic fishes known hitherto were almost entirely middle and upper Triassic in age.

Some of the Triassic fishes are found in marine and some in fresh-water deposits, a matter of importance when comparisons are being made. The principal faunas may be listed as follows:

	Marine	Fresh water
Lower Triassic:	Spitsbergen East Greenland Madagascar	South Africa
Middle Triassic:	Muschelkalk Alpine Trias	New South Wales
Upper Triassic:	Alpine Trias	Newark beds (U.S.A.) Keuper (usually sporadic)

¹ The precise age of the various levels of the Hawkesbury series is a matter of some doubt

II. THE PRE-TRIASSIC PALAEONISCIDAE AND THE HOLOSTEANS

The Palaeoniscidae. The Palaeoniscidae are important in this study, for apart from being themselves represented in the Triassic it is almost certain that all the other bony fishes of the Triassic, with the exception of the coelacanthids and dipnoans, both very minor groups by this time, were descended from the palaeoniscids.

The Palaeoniscidae is usually styled a family, but it has been felt in recent years that this great central mass of the Chondrostei is an assemblage of families which, in some respects, are remarkably uniform in structure (Watson, 1925, 1928). One of the most constant, and from our point of view, one of the most important char-

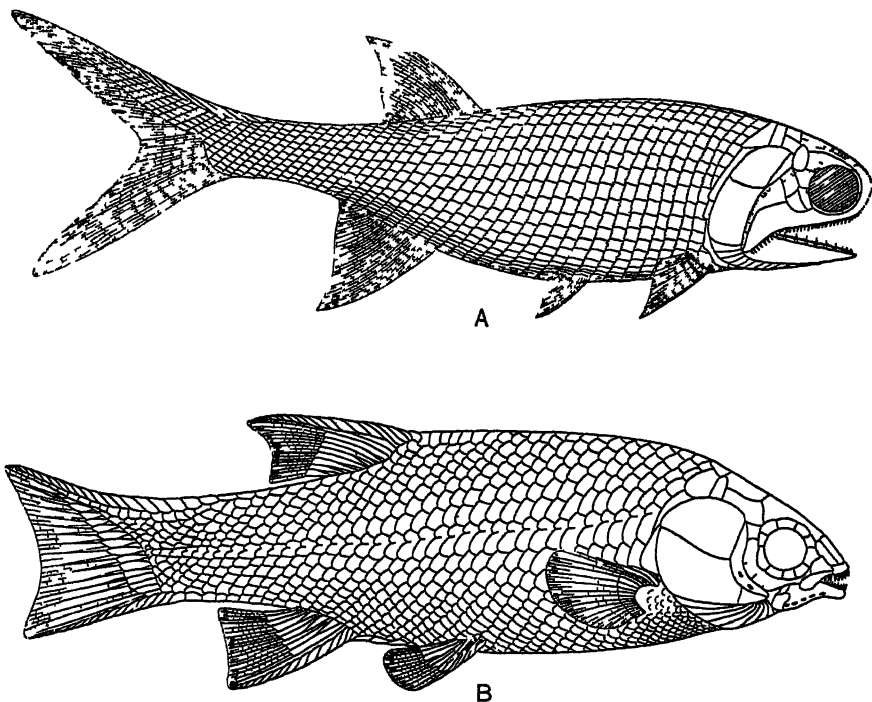


FIG. 1. A palaeoniscid, *Dicellogadus* (A) and a semionotid, *Acentrophorus* (B).
(A after Brough, and B after Gill.)

acters of the group is the nature of the cheek. The preoperculum is large and spreading, and is firmly united to a very deep and prominent maxilla, thus giving in the posterior jaw region a solid bony plate which articulates with the opercular bones (Fig. 1 A).

The palaeoniscids make their appearance in the middle Devonian along with the crossopterygians, but they are from the beginning entirely distinct from the latter group.

They were rare in the Devonian, but came rapidly into prominence in the Carboniferous, and in the Permian period they were the predominant group of

bony fishes. Their rise coincided with the decline of the crossopterygians, which, after Carboniferous times, always played a minor part. Toward the close of the Permian the palaeoniscids appear to have suffered a sharp decline. In the lower Triassic, palaeoniscids are still fairly common, but other groups are well represented, while in the higher Triassic palaeoniscids become rare, the majority of bony fishes then belonging to the Holostean group. After the Triassic, palaeoniscids are found but rarely, and finally die out in the Cretaceous.

Acentrophorus. This genus is represented by a number of small fishes from beds of upper Permian age (Gill, 1923 *a*). It is holostean in every sense and is a somewhat primitive, but on the whole, a typical member of the family Semionotidae.

The immediate ancestors of this fish are unknown, but it was presumed to have been derived from palaeoniscids, a view which incidentally is well supported by the most recent work on the groups derived from the palaeoniscids, for many of these, although not holosteans, show well how the holostean structure may have arisen.

Acentrophorus, a perfect holostean, thus makes its appearance while the palaeoniscids are still the dominant bony fishes, and the theory was put forward that these small semionotids gave rise to new forms as the palaeoniscids declined and, in fact, were the ancestors of the holostean group (Tate Regan, 1904, 1923, 1929).

It is important when dealing with the peculiar sub-holostean families of the Triassic to remember that holosteans proper had been evolved long before and that one is not dealing with the actual origin of the Holostei, although much of the evidence obtained from a study of the Triassic groups shows well how the holosteans were probably evolved.

III. THE BREAK-UP OF THE PALAEONISCIDAE

We know now that the view that the palaeoniscids died out and were gradually replaced by the holosteans which had existed already in Permian time does not quite represent the facts.

We may view the Palaeoniscidae as a major group of fishes composed of a number of independent families. Toward the close of the Permian period many of these groups began to show changes of structure and a replacement of palaeoniscid by holostean characters. These changes were parallel, that is, similar changes took place in independent groups, but the changes proceeded further in some families than in others. The Catopteridae, for example, advanced little beyond the palaeoniscid grade of structure, while the Ospiidae and Parasemionotidae went so far, that it may be difficult to decide whether or not they are to be considered as true holosteans.

The term sub-holostean will be applied to these families. It is not proposed as a classificatory name but is merely one of convenience, and is used here to indicate the several families of fishes which were derived from the Palaeoniscidae but which display certain holostean characters in the tail, fins or head. These families appear in the Triassic and, with one exception, do not appear to have survived that period. Although possessed of certain holostean characters, these fishes are not holosteans,

nor are they the ancestors of the Holostei. They appear to have died out without giving rise to new forms.

The parallel changes undergone by these groups may be listed as follows:

(1) *Reduction of tail.* The tail in palaeoniscid fishes is fully heterocercal, and the conditions of hemiheterocercy and finally homocercy reached in their descendants is produced by a reduction of the scaly body lobe, which in the palaeoniscids extends to the tip of the tail.

In the Triassic sub-holostean families a varied amount of reduction is shown; some forms showing a slight reduction from the full heterocercal condition, while others approach true homocercy.

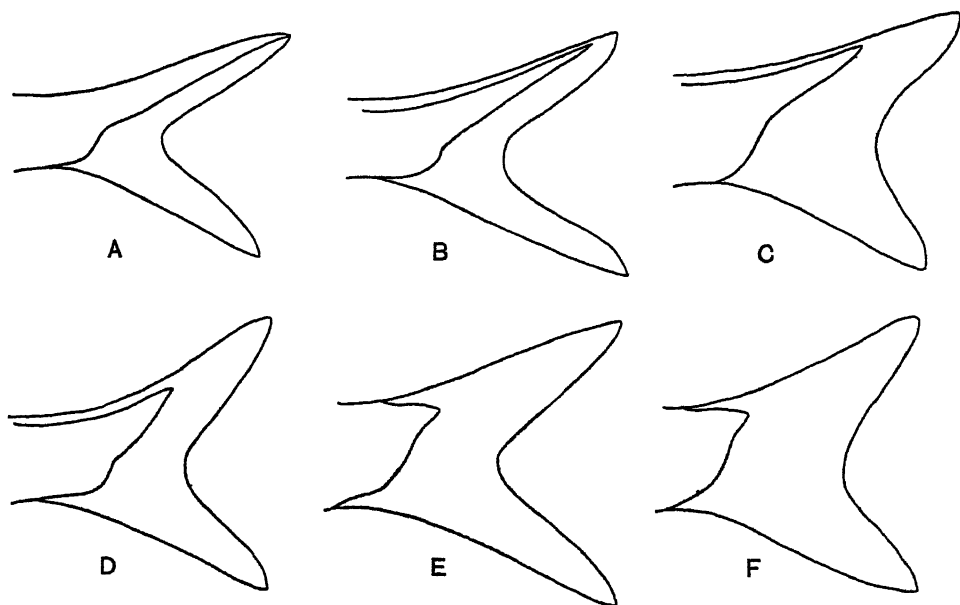


Fig. 2. A palaeoniscid heterocercal tail (A), and a series of catopterid tails, showing the reduction of the body lobe. *Dicelopygae lissocephalus* (A), *Daedalichthys higginsii* (B), *Helichthys obesus* (C), *Helichthys stegopygae* (D), *Catopterus redfieldi* (E), and *Dictyopygae decipiens* (F).

The catopterid family shows a beautiful series of stages in the reduction of the lobe (Fig. 2). In *Daedalichthys* (lower Triassic) the scaly lobe is large and prominent, very much as in palaeoniscids, but in perfect specimens it is seen that the scaly lobe stops just short of the tip of the tail, and that the dorsal lobe of the fin ends in a tuft of short and fine, but otherwise quite ordinary, fin rays. The condition in *Helichthys* (lower Triassic) is variable: in some species the body lobe extends two-thirds of the distance towards the tip of the tail, while in others it is just half. In *Atopocephala*, and in the middle Triassic *Brookvalia* the lobe extends exactly half-way to the tip of the fin. The upper Triassic forms show this tendency being carried still further, and in *Catopterus* the scaly lobe extends scarcely one-quarter of the distance toward the dorsal lobe of the tail. In *Dictyopygae* the lobe is still further

reduced, and is barely visible as such, the tail thus reaching a condition which is closely approaching external homocercy.

The scaly covering of the tail is essentially similar to that of the body and consists of series of longitudinal and transverse rows, and there is evidence suggesting that this scaly lobe may have been reduced in some families by an alternative method to that adopted by the Catopteridae.

Gill has shown that in the Semionotidae the reduction progresses by the removal of longitudinal scale rows from below upwards, and that in the earliest semionotid, *Acentrophorus*, the dorsal-most row of scales is still complete and extends to the tip of the tail (Fig. 3 B). Gill also showed that in a very young *Dapedius* this row was still prominent and extended about two-thirds of the way to the tip (Gill, 1923 a).

It is clear that the alternative method was adopted in the Catopteridae, and that the lobe was reduced by the loss of transverse rows of scales from the tip of the tail forwards. It is due to this method, in which the first and all subsequent losses of scales directly affects the length of the lobe, that such a series of tails from the

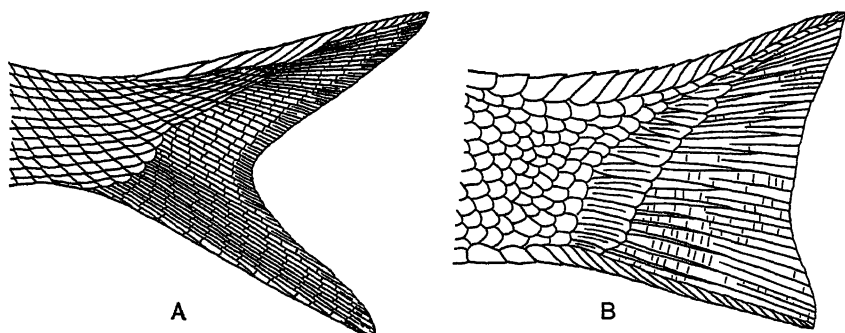


FIG. 3 The tails of *Helichthys elegans* (A) and *Acentrophorus varians* (B) to illustrate the two different methods of reducing the scaly lobe of the tail. (B after Gill)

almost perfect heterocercal to the almost complete homocercal condition can be observed in the Catopteridae. No other group shows the intermediate condition of the scaly lobe, the true hemiheterocercal form, so well.

Tails reduced in the semionotid fashion would not show the intermediate condition in the same way, since they are really heterocercal until the last and the dorsal-most row of scales is removed. When this row does finally disappear there is a comparatively sudden jump from complete to a very much reduced heterocercy.

(2) *Straightening the suspensorium.* The move toward the straightening of the suspensorium constitutes one of the clearest trends affecting the bony fishes in late Permian and Triassic time. It was during this period that the backwardly directed suspensorium, which was the mode in pre-Permian and early Permian times, was replaced gradually by a forwardly directed suspensorium. This modification was accompanied by other profound changes, and is, I believe, correlated with the introduction of the interoperculum which was made necessary by the relative forward movement of the posterior parts of the jaws and their eventual reduction in size.

Although all palaeoniscids possess backwardly directed suspensoria, it is noticeable that in the Triassic genera of the group, the angle of deviation from the vertical is usually not great.

A morphological series can be constructed from the catopterid forms showing the change from a definitely oblique to a vertical suspensorium. The condition in the middle Triassic *Brookvalia* is essentially that of the palaeoniscids: the suspensorium is oblique and backwardly directed. In *Daedalichthys* it is only a little less oblique, while in the other lower Triassic forms, *Atopocephala* and *Helichthys*, it is still backwardly directed but only to a slight degree. In *Catopterus* (upper Triassic) the suspensorium has practically attained the vertical position, while in *Dictyopygae* it is vertical or, in some individuals, slightly forwardly directed.

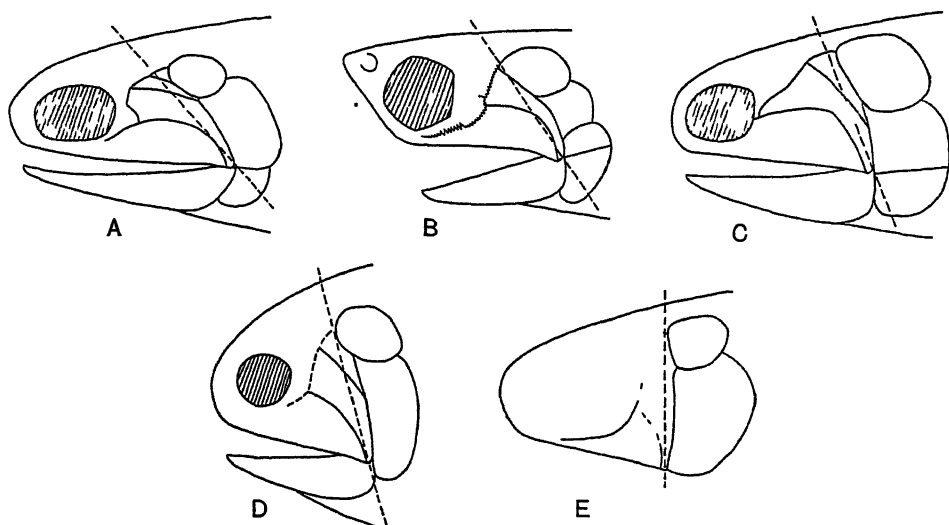


Fig. 4. A series of catopterid skulls showing the swinging forward of the suspensorium. *Brookvalia gracilis* (A), *Daedalichthys hugginsi* (B), *Helichthys elegans* (C), *Catopterus redfieldi* (D), and *Dictyopygae dectpiens* (E). (A after Wade.)

The Perleididae also progressed from the backwardly directed to a forwardly directed suspensorium. In the lower Triassic species of *Perleidus* described by Stensiö (Stensiö, 1921), the suspensorium is slightly backwardly directed but upper Triassic species had a vertical suspensorium. The middle Triassic genera of perleidids described by Wade appear to have the suspensorium about vertical.

A similar forward movement of the suspensorium almost certainly occurred in the other sub-holostean families, but in these, intermediate stages are not known.

(3) *Modifications of fin rays.* The modifications of the fin rays was another great change which appears to have affected many groups of the actinopterygian fishes simultaneously. Primitively the rays are fine and numerous and each is broken up by transverse joints into several pieces. The tendency in both paired and unpaired fins was toward reduction, until the external fin rays coincided in number with the endoskeletal supports. The modification appears to proceed successively from the

anterior to the posteriorly placed fins. The pectoral fins are affected first, then the pelvic and finally the unpaired fins. The caudal is usually the last fin to be modified.

Some of the Triassic palaeoniscids show the earliest stage in this series of changes; the anterior one or two rays of the pectoral fin being stiff and unjointed except at the tip (*Diaphoragnathus*). There is, however, the peculiar Carboniferous palaeoniscid, *Phanerorhynchus ornatus* (Gill, 1923 b) in which all the fins that can be seen are of the modern type with few stiff unjointed rays. This was a precocious development, but it is not uncommon to find modifications thus heralded long before their proper phase arrives. It was a premature evolutionary birth; the main movement coming later, in Permian and early Triassic times.

On the whole, the catopterids did not progress very far beyond the palaeoniscid condition. The paired fins are usually modified to a greater or less extent, while the unpaired fins are unmodified. In the most extreme forms the rays of the pectoral and pelvic fins are very few in number, perhaps only six or seven, and each is large, stiff and unjointed. A morphological series can be arranged to show the lines along

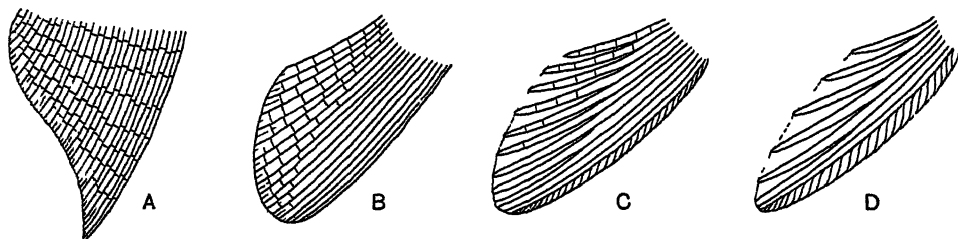


Fig. 5. A palaeoniscid pectoral fin (A), and a series of catopterid fins (B-D) showing the transition from the many and slender rayed to the few and stout rayed condition. *Helichthys obesus* (B), *Helichthys grandipennis* (C), and *Daedalichthys higginsii* (D).

which the catopterid pectoral fin has been modified. The palaeoniscid condition is not shown in any of the catopterid forms, but certain species of *Helichthys* approach it very closely. In *Helichthys obesus*, for example, the appendage is essentially palaeoniscid, except that the base is very slightly constricted and that the more anterior rays have lost their articulation. Otherwise, it consists of numerous fine articulated rays, and lacks fulcra. In *Helichthys grandipennis* the fin base is constricted, the rays are few and stout, but the posterior ones are still articulated; fulcra are prominent. In *Daedalichthys higginsii* the highest degree of modification is attained. The base is extremely short; there are very few rays which are, however, stout and completely lack articulations, while the anterior border of the fin is fringed by a series of massive fulcra (Fig. 5).

The lack of correlation of structure between the pectoral and pelvic fins is well shown in some of the forms mentioned above. In *Helichthys grandipennis*, for example, while the pectoral fin is given as an example of one of the highly evolved types, the pelvic fin remains in the primitive palaeoniscid condition. In other catopterids, however, the structure of the pectoral or pelvic fins may be identical.

In the series given above there is no evidence that reduction in number of rays was effected by a bunching together of fine rays. This latter method was, however,

probably the one usually adopted. In the Ospiidae, Parasemionotidae and Semionotidae the fin rays equal in number the endoskeletal supports, but while each is a single structure proximally, they branch distally to a few fine rays. It is assumed, therefore, that in these forms the reduction was brought about by, first, a bunching, and later, a fusion of the fine rays.

In the Pholidopleuridae, while the posterior rays of the dorsal and anal fins may equal in number the endoskeletal supports, the anterior rays are crowded together and clearly outnumber the supports (Stensiö, 1932; Piveteau, 1935; Wade, 1935). In the Cleithrolepididae the same condition is observed, but the general condition is one of equality between basalia and external rays, only a few fine closely set rays occurring at the anterior end.

It is possible that alternative methods of fin reduction have been followed by the Triassic sub-holostean families; either by bunching and fusion, or by enlargement of certain individual rays and eliminations of the rest. It must be pointed out, however, that the second form of reduction has only been observed in the paired fins (Catopteridae), never in the unpaired fins.

(4) *Scale structure.* The typical palaeoniscid scale consists of three layers—a mass of lamellated bone below, ganoine on the upper surface and an irregular layer

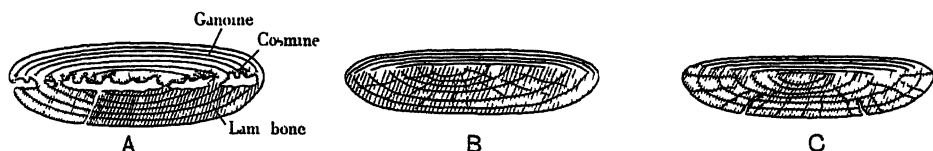


Fig. 6. A typical palaeoniscid scale (A), and catopterid (B) and lepidosteid (C) scales both showing the loss of the cosmine layer. (A and C after Goodrich.)

of cosmine between (Goodrich, 1907). In the holostean the scale consists of lamellated bone and ganoine, the cosmine being lost, a condition well shown in the scales of the living *Lepidosteus*. In certain of the sub-holostean families in which the histology of the scale has been studied, the same change is found to have taken place. In the Catopteridae (Brough, 1931) the cosmine layer is absent, and in the Perleididae (Stensiö, 1932) this layer is generally absent, though vestiges may be present (Fig. 6). In the Ospiidae the scales, according to Stensiö (Stensiö, 1932), are of the lepidostean type.

(5) *Modification of the sensory canals.* The sensory canals in the heads of palaeoniscids usually pursue a definite and unvaried course. The main cephalic divisions of the canal comes up from the posterior part of the head, runs forward as far as the orbit and then turns sharply downward to form the infra-orbital canal. The supra-orbital canal runs in the frontal bone above and does not have any posterior connection with the infra-orbital canal (Fig. 7).

In the Holostei the supra-orbital canal joins the main cephalic division where it turns down as the infra-orbital canal. This change over from the first to the second type, which can be seen admirably in the development of *Amia* (Allis, 1889), has been independently achieved at least three times (Fig. 7). Watson has figured a head of *Oxygnathus ornatus* (Watson, 1925), a lower Liassic palaeoniscid in which

the infra-orbital and supra-orbital canals are joined. Wade has shown that the Australian catopterid genus *Brookvalia* had a similar arrangement (Wade, 1935). Neither of these had any connection with the semionotid stem in which the same modification was carried out.

In most of the Triassic sub-holosteans, Catopteridae (except *Brookvalia*), Perleididae, Ospiidae, Parasemionotidae, Cleithrolepidae, Pholidopleuridae, Bobasatranidae, the sensory canals of the head were arranged on the normal palaeoniscid plan.

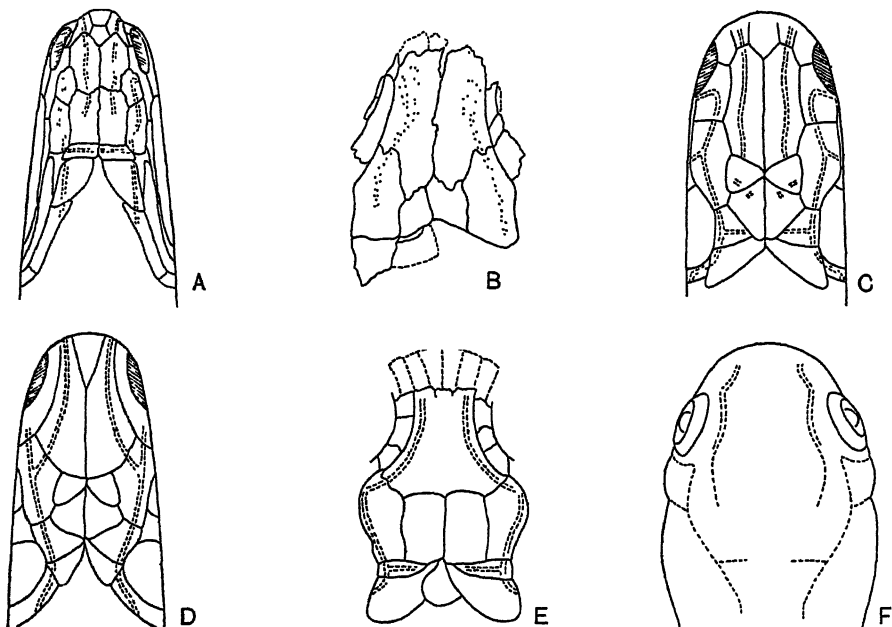


Fig. 7. A series of heads showing three independent lines in which the supra-orbital and infra-orbital sensory canals have become connected behind the orbit. *Cheirolepis trailli* (A), showing the normal palaeoniscid condition, and *Oxygnathus ornatus* (B), a palaeoniscid in which fusion has occurred. *Helichthys elegans* (C), a catopterid showing the typical palaeoniscid condition, and *Brookvalia* (D), a catopterid showing secondary fusion. *Acentrophorus varians* (E), an early holostean with the canals joined and a larval *Ania* (F) at an early stage in development still showing the independence of the supra-orbital and infra-orbital canals. (A and B after Watson, D after Wade, E after Gill, and F after Allis.)

(6) *Reduction of the maxilla*.¹ Modifications such as those listed above are all tending in the direction of holostean structure, but many groups of fishes underwent these modifications to a greater or less degree without becoming holosteans. The character to be considered now, however, is a critical one, and any fish in which the maxilla is markedly reduced and is free of the preoperculum must be close indeed to the Holostei.

This change appears to have been initiated at least three times:

(a) In the Semionotidae, where it was completely carried through and true holosteans evolved.

¹ The free maxilla was also acquired in the peculiar Permian genus *Dorypterus* (Gill, 1925).

(b) To some extent in the Ospiidae (Stensiö, 1932), where although the maxilla is not present in the type specimen its detached condition is inferred by the lack of a palatal flange for its attachment to that structure, a condition seen in the palaeoniscids; the rounded nature of the anterior edge of the preoperculum which does not appear to have articulated with anything; and the presence of a pronounced coronoid process on the mandible which gives this structure a holostean appearance.

(c) To a greater extent in the Parasemionotidae (Piveteau, 1935) where the maxilla can be seen somewhat reduced from the palaeoniscid condition and entirely free of the preoperculum. The latter bone is also considerably reduced and an interoperculum is developed.

According to Stensiö the last two families are entirely independent of one another (Stensiö, 1932).

IV. THE SUB-HOLOSTEAN FAMILIES

We now have the conception of the Palaeoniscidae toward the close of Permian times consisting of a number of independent subgroups, many of which began to show changes of structure towards the holostean condition. These changes were carried out independently and at unequal speeds, some progressing much further than others toward the holostean grade of structure.

It is proposed here to give a list of the families derived from the Palaeoniscidae and to indicate how far each has evolved in the holostean direction. (For detailed definitions of these families see Brough (1931), Stensiö (1932), Piveteau (1935), Wade (1935).)

It must be stressed here that each of these subgroups had its own special characters which have not yet been considered but which will be briefly listed below. The characters noted in the previous section were parallel, and common to more than one group.

(1) *Catopteridae* (Brough, 1931, 1934; Wade, 1935). This is the least modified of the sub-holostean groups. The tail has been reduced from the heterocercal condition and the rays of the paired fins have been reduced, but otherwise they closely resemble the palaeoniscids.

In one member, *Brookvalia*, the sensory canals of the head have been modified from the palaeoniscid to the holostean form by the union of the supra- and infra-orbital canals. The scales are modified from the palaeoniscid to the holostean type by the loss of the cosmine layer (only one genus, *Catopterus*, has been examined in this respect).

Special characters: shape and arrangement of the skull bones; absence of branchiostegal rays.

This family ranged throughout the Triassic and consists of the following genera—*Daedalichthys*, *Helichthys*, *Atopocephala* (lower Triassic South Africa, Brough, 1931, 1934); *Brookvalia*, *Beaconia*, *Dictyopleurichthys*, *Gertonichthys*, *Molybdichthys*, *Phlyctaenichthys*, *Schisorichthys* (middle Triassic New South Wales,

Wade, 1935), *Catopterus*, *Dictyopygae* (upper Triassic North America, Brough, 1931)

(2) *Perleididae* (Stensio, 1921, 1932, Brough, 1931, Piveteau, 1935, Wade, 1935) In these fishes the tail is reduced to the hemiheterocercal condition, and the fin rays are reduced so that the external rays equal the endoskeletal supports in the unpaired fins. The suspensorium varies from slightly backwardly to slightly forwardly directed.

The scale structure approaches the lepidostoid type, but traces of a cosmine layer still remain (Stensio, 1932). The sensory canals of the head are of palaeoniscid pattern, as are the bones of the cheek and jaws.

Special characters arrangement of the skull bones, teeth styliform.

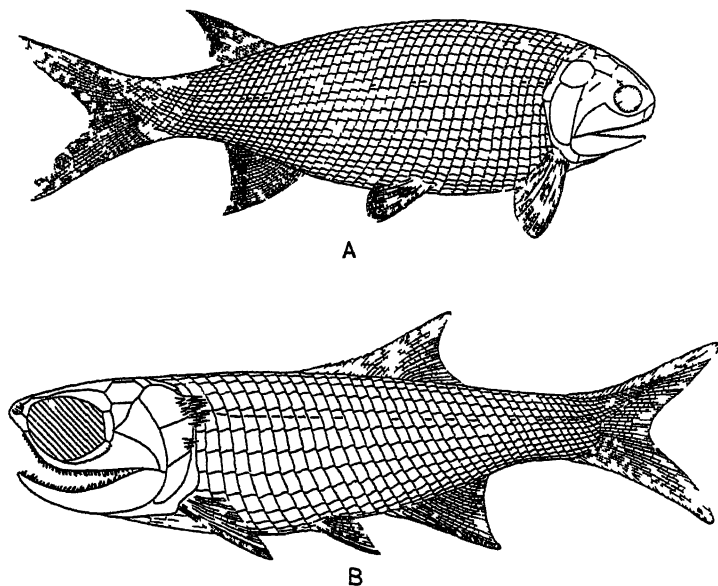


Fig 8 Two catopterid fishes, *Helichthys* (A) and *Catopterus* (B) (Both after Brough)

Fishes of this family are found all through the Triassic, the following genera are known: *Perleidus* (lower and middle Triassic, Stensio, 1921, 1932), *Meiduchthys* (lower Triassic, Brough, 1931), *Manhetta*, *Procheuichthys* (middle Triassic, Wade, 1935), *Colobodus*, *Dollopterus*, *Meridensia* (European middle and upper Triassic).

(3) *Cleithrolepididae* (Brough, 1931, Wade, 1935) Tail reduced to hemiheterocercal, rays of unpaired fins reduced to coincide with endoskeletal supports except in anterior region of dorsal and anal fins (*Cleithrolepis*). Suspensorium vertical or forwardly directed. Histology of the scales not known. Sensory canals of the head developed on palaeoniscid pattern. Cheek region and jaws as in the palaeoniscids.

Special characters extremely deep bodied and laterally flattened, mandible weak and shelf-like, teeth minute or absent, arrangement of skull bones.

The genera are *Hydropessum* (lower Triassic South Africa) and *Cleithrolepis* (lower Triassic South Africa, middle Triassic New South Wales). Wade has

included *Dipteronotus*, Egerton, in this family, but the structure of this genus is incompletely known

(4) *Bobasatramidae* (White, 1932, Stensio, 1932) An interesting family in which the caudal fin is still completely heterocercal, but in which the rays of the dorsal and anal fins approximately equal the number of the endoskeletal supports

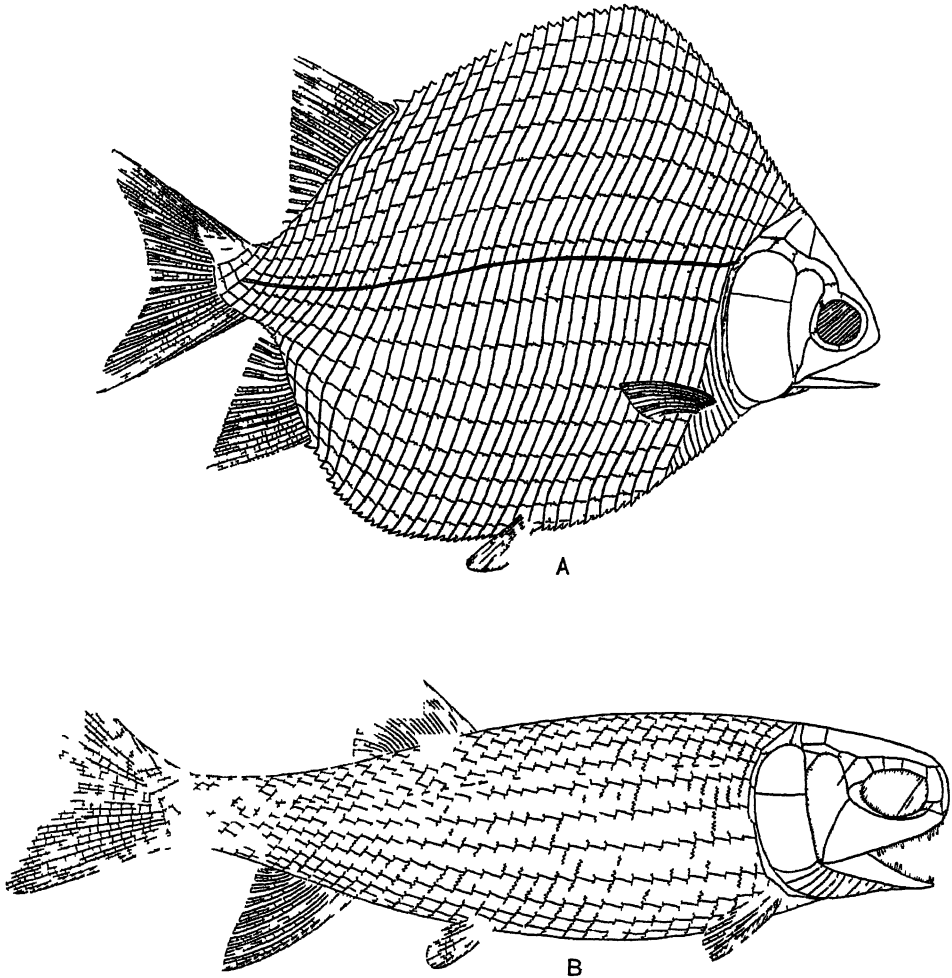


Fig 9 (A) *Cleithrolipus* (Cleithrolepidae), (B) *Meioduchthys* (Perleididae) (Both after Brough)

(Stensio, 1932) Suspensorium vertical or sloping slightly backwards Scale structure resembling that of *Platysomus*, a slightly modified palaeoniscid type Sensory canals of the head as in palaeoniscids Cheek and jaw region not well known

Special characters extremely deep bodied and laterally flattened, rhombic in shape, opercular apparatus much modified (Stensio, 1932)

It consists at present of only one genus, *Bobasatrania*, from the lower Triassic of East Greenland and Madagascar, but it is likely that *Ecrinosomus* (lower Triassic, Madagascar) belongs to the same family.

(5) *Ospiidæ* (Stensiö, 1932). This most interesting family is unfortunately known only from incomplete remains. The caudal fin is not known. In the unpaired fins the external rays equal the endoskeletal supports. The suspensorium is about vertical. The scales are of lepidosteid type, without the layer of cosmine found in the palaeoniscids. Sensory canals of the head as in palaeoniscids. The cheek and jaws show an advance on the palaeoniscid condition. The preoperculum is slightly reduced in size with a rounded front margin, it did not appear to articulate with the maxilla, which was at least partly free. The mandible developed a strong coronoid process.

Special characters: shape and arrangement of head bones.

Only two genera, *Ospia* and *Broughia*, are known, both from the lower Triassic of East Greenland.

(6) *Parasemionotidae* (Piveteau, 1935). The tail is hemiheterocercal, but the scaly lobe is still rather long in *Watsonia*. In the unpaired fins the external rays equal in number the endoskeletal supports. The suspensorium is vertical or slightly backwardly directed. Internal structure of the scales not known. Sensory canals of the head are of a palaeoniscid pattern. Bones of the cheek and jaws approaching the holostean condition. The preoperculum is reduced, markedly so in *Parasemionotus*, and is not attached to the maxilla, which is a less stout bone than that in the palaeoniscids, the posterior portion being much reduced.

Special characters: arrangements of bones of cranial roof; an interoperculum is developed.

Only two genera are known, *Parasemionotus* and *Watsonia*, both from the lower Triassic of Madagascar.

(7) *Pholidopleuridae* (Wade, 1932, 1935; Stensiö, 1932; Piveteau, 1935). The Pholidopleuridae show an extraordinary mixing of primitive and advanced characters. The tail is completely homocercal and is thus in a much more advanced condition than in any of the foregoing groups, but the fins have the primitive palaeoniscid appearance with many fine rays which outnumber the endoskeletal supports. The suspensorium is generally oblique and backwardly directed, as in the palaeoniscids, but in *Pholidopleurus* (upper Triassic) it is almost vertical. According to Stensiö the scales (*Australosomus*) have lost both the ganoine and cosmine layers and consist essentially of lamellated bone (Stensiö, 1932, p. 181). The sensory canals of the head are as in palaeoniscids, but the supra-orbital canal does not extend back to the parietal but ends further forward in the frontal bone. The cheek region and jaws are exactly of palaeoniscid type.

Special characters: the squamation is modified, there being rows of enlarged scales along the flank; ossified vertebral centra are present; clavicles absent as separate elements.

The family is represented by the following genera: *Australosomus* (lower Triassic of East Greenland and Madagascar), *Macroaethes* Wade (middle Triassic

New South Wales), and *Pholidopleurus* Bronn (Alpine Trias), and thus ranges throughout the whole of the Triassic.

(8) *Saurichthyidae*. This peculiar and highly specialised family has been variously placed in the fish classification by different authors, some regarding it as a chondrosteian, others as a holostean family. Its relationship to the palaeoniscids was first established by Smith Woodward (Woodward, 1890) and this view was subsequently upheld by Stensiö in his detailed paper on the Spitsbergen saurichthyids (Stensiö, 1925).

The scaly lobe of the tail is entirely reduced, and the tail is in a condition which has usually been described as abbreviate diphyccercal. The fin rays are fine and numerous, and easily outnumber the endoskeletal supports. The suspensorium is about vertical. The squamation is reduced to four rows of scutes. The sensory canals of the head are of palaeoniscid type, but the supra-orbital canal does not extend far back in the frontal bone. The cheek and jaw regions, although much modified, still retain their essential palaeoniscid character. The preoperculum is extensive and is firmly united to the maxilla.

Special characters: the anterior region of the head is drawn out into a long beak; squamation reduced to four isolated longitudinal rows; notochord, although persistent, is invaded by processes from the base of the neural arches.

These fishes range from the lower Triassic into the Jurassic. The systematic position of the various members of the family is unsettled (Stensiö, 1925); Stensiö recognises the genera *Saurichthys* and *Acidorrhynchus*.

The Semionotidae. The foregoing eight families have been termed in this paper the sub-holostean groups, and are fishes derived from the palaeoniscids and all changed more or less toward the holostean grade of structure. Some (catopterids) have the palaeoniscid impress strongly upon them, others (*Parasemionotidae*) can scarcely be distinguished from the holosteans. In order to give a basis for comparison the *Semionotidae*, the most primitive family of the holosteans, will be treated here in the same way as the previous groups.

Tail hemiheterocercal, with the scaly lobe usually much reduced. Fin rays relatively few and equalling in number the endoskeletal supports of the dorsal and anal fins. Suspensorium usually slightly forwardly directed. Scales consisting of a lower layer of lamellated bone and an upper layer of ganoine. Cosmine layer characteristic of the palaeoniscids, absent. The supra-orbital and infra-orbital sensory canals of the head are united behind the orbit. The maxilla is much reduced and is entirely free of the preoperculum which is also reduced and now appears as a narrow crescent of bone.

An interoperculum is introduced, and clavicles are absent.

The neurocranium. A good deal of valuable work has been done on the neurocranium of the *Perleididae*, *Ospidae* and *Pholidopleuridae* (Stensiö, 1932; Piveteau, 1935). It has been found in all cases that the neurocranium shows a marked resemblance to that of the *Palaeoniscidae* as seen in the palaeoniscid crania A and B described by Watson (Watson, 1925, 1928), and is certainly not of a holostean type.

V. THE ORIGIN OF THE HOLOSTEI

Although it has long been recognised that the Palaeoniscidae were probably ancestral to the Holostei, the two groups have always been sharply defined. The first holostean, *Acentrophorus*, shows few signs of its palaeoniscid ancestry. It already possesses stout fin rays equalling in number the endoskeletal supports, an interoperculum, reduced preoperculum and maxilla, and the posterior freedom of the latter bone. It does display a single row of body scales reaching the tip of the tail, a relic of heterocercy in its ancestors.

The Triassic sub-holosteans provide a series of links which bridge the gap between the chondrosteian and holostean grades of structure. Certain of the catopterids are completely palaeoniscid except that the scaly lobe of the tail is reduced. The typical perleidid is a fish with a palaeoniscid head and a holostean body; the lobe of the tail is reduced, and the number of rays in the fins is reduced to equal the endoskeletal supports, but the skull is essentially that of a palaeoniscid. In the Parasemionotidae the body is like that of the perleidids but in this group the skull is also changed. A small interoperculum is present, the firm connection between the maxilla and the preoperculum is broken and these bones are reduced. This leads on directly to the full holostean structure as seen in the Semionotidae.

Two points must be made clear at this stage. The series above is only morphological; the catopterids, perleidids and parasemionotids are separate groups which independently show the changes toward the Holostei. Also, the Holostei were already in existence before the beginning of the Triassic period, the solitary genus *Acentrophorus* being known from rocks of Permian age.

The sub-holostean families dealt with in this paper could not, therefore, have given rise to the Semionotidae, the first of the holosteans, but whether any of them persisted and gave rise to other holostean families is a question only to be answered by future research.

VI. THE EVOLUTION OF THE CATOPTERIDAE

Each of the sub-holostean families dealt with above was an independent unit, and it has already been shown that on analysis, two different sets of characters can be distinguished: (1) those which were parallel and which were also being carried out in other families; (2) those which were peculiar to the individual family.

The first group has already been considered, but while these major changes were proceeding minor characters may also have been undergoing modification. The Catopteridae may be considered from this point of view, since it is one of the best known of the sub-holostean group, and ranges from the lower to the upper Triassic. Certain changes of the second type can be seen in this group and may be listed as follows:

(1) *Reduction in the size of the orbit* (Fig. 10). The large-eyed character of the catopterids is more prominent in the primitive forms, particularly in *Atopocephala*, in which the orbit is comparatively enormous. It is only a little less conspicuous in

Daedalichthys, and in the middle Triassic, *Brookvalia*, although smaller than in the former genera it is still large. *Helichthys* shows a further reduction in the size of the orbit, and finally in the upper Triassic *Catopterus* it is comparatively small.

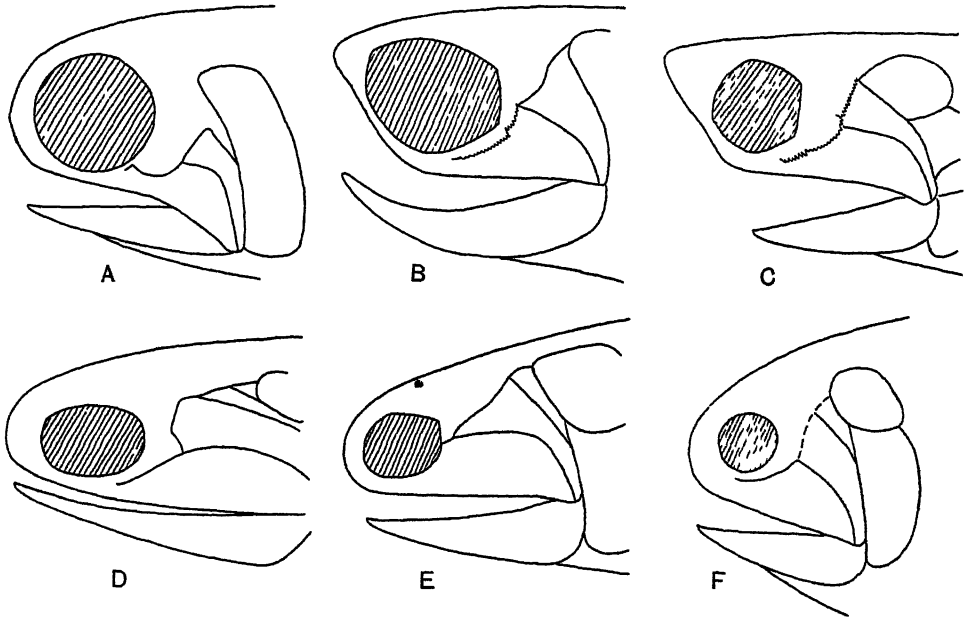


Fig. 10. A large-eyed palaeoniscid, and a series of catopterids showing successive reduction in the size of the orbit. These were all small fishes varying very little in length and proportions. The skulls are drawn to a standard size. *Dicelopygia lissocephalus* (A), *Atopocephala watsoni* (B), *Daedalichthys hugginsi* (C), *Brookvalia gracilis* (D), *Helichthys elegans* (E) and *Catopterus redfieldi* (F). (D after Wade.)

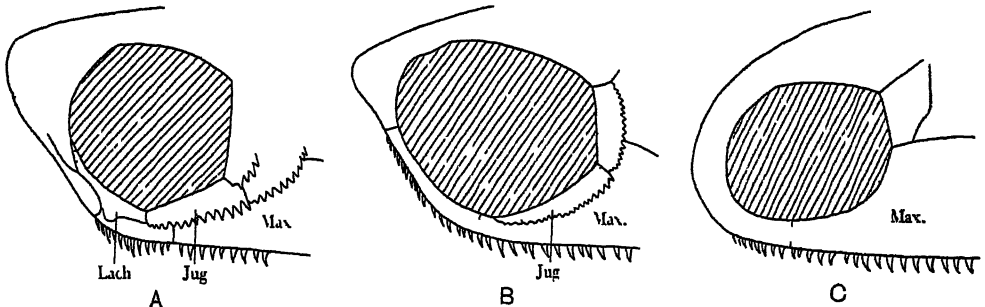


Fig. 11. A series of catopterids showing the reduction of the sub-orbital bones. *Daedalichthys hugginsi* (A), *Atopocephala watsoni* (B), and *Helichthys elegans* (C).

(2) *Loss of the sub-orbital bones* (Fig. 11). This is only seen in the South African catopterids, but it is one of the clearest trends to be observed in the family. The condition of *Daedalichthys* is in this respect the most primitive. The orbit is bordered above and in front by the post-frontal and the nasal, and behind and below by the post-orbital, jugal, and lachrymal. The jugal and lachrymal are fairly prominent and lie above the maxilla and premaxilla, being interposed between these bones and the orbit.

In *Atopocephala* the reduction of these elements has begun apparently proceeding from in front. The posterior part of the jugal is as in *Daedalichthys*, but it is reduced anteriorly where it tapers away to a point. The reduction of the lachrymal has gone further, and it is either completely absent or reduced to a very thin selvage of bone, so that practically the anterior end of the maxilla and the greater part of the pre-maxilla enter the orbital rim. The trend is completed in *Helichthys* where both lachrymal and jugal are absent, and part of the posterior and almost the whole of the lower border of the orbit is formed by the maxilla.

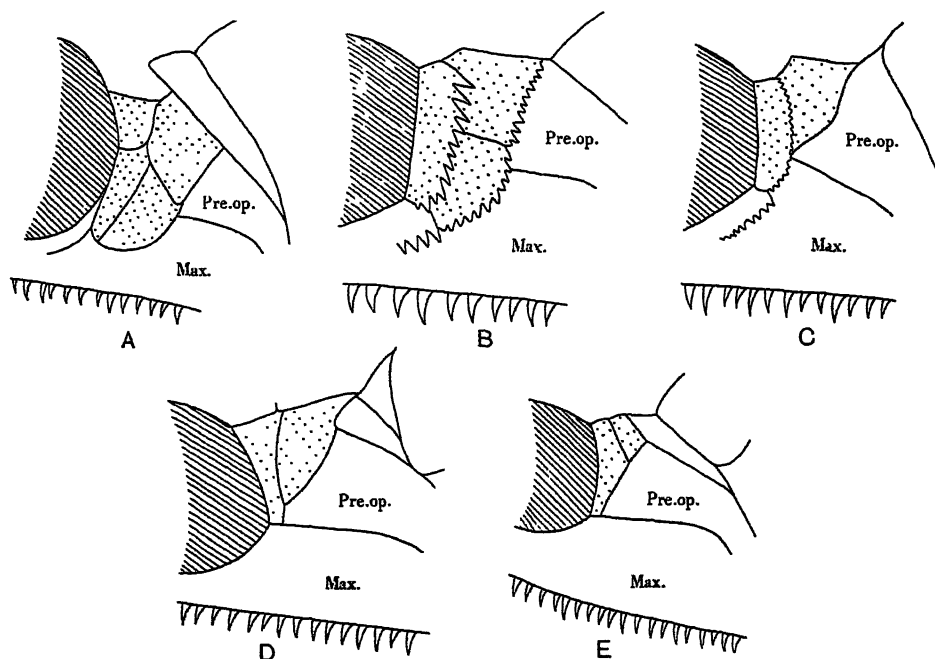


Fig. 12. The palaeoniscid *Dicelopygae* and a series of catopterids showing the reduction of the post-orbital bones. *Dicelopygae lissocephalus* (A), *Daedalichthys higginsii* (B), *Atopocephala watsoni* (C), *Helichthys ctenipteryx* (D), and *Helichthys obesus* (E).

(3) *Modification of the post-orbital bones* (Fig. 12). In *Daedalichthys* there is a large post-orbital bone, and immediately posterior to this and in front of the pre-operculum there are two others which, for convenience, are called the second row of post-orbitals. In *Atopocephala* and *Helichthys* this second row is not nearly so prominent, consisting of a single bone. This is a large element of fair depth in *Atopocephala*, but in *Helichthys* the depth is reduced and the bone consequently appears to be more elongated in an antero-posterior direction. It is in front comparatively smaller than the same element in *Atopocephala*, and it reaches its minimum in *Helichthys obesus*, where it is a tiny element.

All the post-orbital bones in *Daedalichthys* have their posterior margins pectinated, the post-orbital proper, very markedly so. In *Atopocephala* the single second post-orbital has lost its pectination, the posterior border being perfectly smooth. The post-orbital proper still retains a serrated posterior margin, which is,

however, less conspicuous than that of *Daedalichthys*. Finally, in *Helichthys* the margin of both post-orbital bones is quite smooth, the pectination of the posterior border having completely disappeared.

The origin of the Catopteridae. Although it has been shown that a number of independent sub-holostean families existed during the Triassic period, and it can be demonstrated that these fishes had their origin in the Palaeoniscidae, it is as yet possible only in one case to link up a sub-holostean group with its palaeoniscid ancestors. This group is the Catopteridae, and the reasons are set down below for presuming it to have been derived from the dicellopygid palaeoniscids.

The earliest catopterids known are those from the lower Triassic fish bed at Bekker's Kraal (Brough, 1931), and in the same bed there are remains of palaeoniscid fishes of the genus *Dicellopygae*. There is a large measure of superficial resemblance between these early catopterids and the dicellopygids; they were small predaceous fishes with long jaws armed by numerous sharply pointed teeth, but these superficial resemblances may mean much or little; a consideration of the structure of the skulls furnishes more positive evidence.

The very large size of the orbit in certain of the catopterid fishes is a striking characteristic, and it is significant that the only known contemporary fishes with an orbit which is at all comparable are the dicellopygids. The relative position of the orbit is also similar; it lies well forward and occupies the greater part of the anterior half of the head. The size of the orbit in *Dicellopygae lissocephalus* is just about the same as that of *Atopocephala watsoni*, the largest eyed catopterid. The value of this comparison is added to by the fact that these fishes are almost exactly the same size.

The bone called post-frontal in the catopterids is an element of large size and peculiar relationships. It lies on the flank of the frontal bone, and its outer margin forms almost the whole of the dorsal border of the orbit. It becomes deeper posteriorly and is in contact with the supratemporal. Its posterior portion appears to take the place of the bone called intertemporal by Watson and the downward bend of the infra-orbital sensory canal which is frequently in the intertemporal in palaeoniscids (Watson, 1925) is, in the catopterids, confined to this large "post-frontal" bone.

The "post-frontal" of *Dicellopygae* is of the same form and has similar relationships as the bone of that name in the catopterids, where it is also the site of the downward bend of the infra-orbital sensory canal.

The bones of the skull roof of *Dicellopygae* show a close similarity to those of the catopterids, particularly to those of *Daedalichthys* and *Atopocephala*. The frontals, parietals, tabulars, post-temporals and post-frontals are all comparable, and there is in fact a much greater degree of resemblance between *Dicellopygae* and *Daedalichthys* in the structure of the skull roof, than between *Dicellopygae* and any other palaeoniscid with which I am acquainted.

The condition in the Palaeoniscidae of the small bones immediately posterior to the orbit (between the orbit and the preoperculum) is extremely variable. The arrangement appears to be haphazard and is usually different in each genus. The

dicellogygid type consists of four small bones set in two rows and more or less equal in size, giving the effect of an intersected square. A fusion of the most anterior pair would produce the pattern of post-orbital ossicles seen in *Daedalichthys*, which is also the most primitive arrangement known in the Catopteridae. This character is of importance, particularly since the tendency in the catopterids is toward the reduction of these post-orbital bones, and *Dicellogyge* provides an admirable basal member for the series (Fig. 12).

This close similarity in the structure of the skull bones provides very strong reasons for supposing that there is a connection between these two groups, and it must be concluded that the catopterids were either evolved from a pre-Triassic species of *Dicellogyge* itself or from some other closely related genus.

It is a notable fact that all the catopterid remains found up to the present have been obtained from freshwater deposits, and the present writer (Brough, 1931) has already tentatively suggested that the group may have been evolved in fresh water, possibly on, or near, the African land mass.

VII. SUMMARY

The Palaeoniscidae was the dominant group of bony fishes during Carboniferous and Permian times. This large and varied group of fishes has usually been called the "family Palaeoniscidae", but it appears to consist of several independent lines.

The Holostei were evolved from the Palaeoniscidae before the beginning of the Triassic, *Acentrophorus*, the earliest member, appearing in the upper Permian.

About the close of the Palaeozoic era the Palaeoniscidae gave rise to several independent families which become modified to a greater or less extent toward the holostean grade of structure.

These groups are referred to as the sub-holostean families. The parallel modifications they display include the reduction of the scaly lobe of the tail, reduction of the fin rays, loss of the cosmine layer of the scales, and the swinging forward of the suspensorium. The modifications proceeded much further in some groups than in others.

It is shown that although these Triassic families did not give rise to the Holostei, they provide a perfect set of intermediate forms between the palaeoniscid and the holostean condition.

One of the sub-holostean families, the Catopteridae, is analysed and its evolving characters separated into those which are parallel (also undergone by other groups) and those which are characteristic of the family.

Reasons are set out for supposing the Catopteridae to have been derived from the *Dicellogyge* group of the Palaeoniscidae.

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CEPHALIC SUTURES AND THEIR BEARING ON CURRENT CLASSIFICATIONS OF TRILOBITES

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I. INTRODUCTION

NEARLY a hundred years ago trilobite genera were first united into families by Milne Edwards (1840) and by Burmeister (1843). With the progress of discovery the concepts of these families changed and new families were recognised. Major ordinal groupings based mainly on single morphological units were propounded and subsequently abandoned chiefly because the chosen units were progressive characters subject to the processes of parallel evolution. Inherent difficulties are apparent in the construction of a phylogenetic classification of an extinct arthropod group when one must rely solely on the partially known exoskeletal structures of the animals and when access to a knowledge of the details of their nervous systems is denied and only very limited information is available concerning the individual's early life history and its organs of locomotion, feeding and respiration.

Epitomes of the early classifications have been provided by Barrande (1852) and by Beecher (1897). Towards the close of the nineteenth century, Beecher elaborated a classification scheme, of which the germs were conceived by Emmrich (1839) and subsequently modified by Salter (1864), in which trilobite families were combined into groups according to the form of some of the cephalic uncalcified lines or sutures. To this scheme Beecher adduced support from phylogenetic speculation based, as he thought, on firm ontogenetic evidence. It is felt that this classification may best be discussed after treating with the various cephalic sutures.

II. THE CEPHALIC SUTURES AND THEIR DISTRIBUTION

The trilobite cephalic shield, like the thoracic segments and the pygidium, has marginal ventrally reflexed extensions developed to a greater or less degree, and these extensions are termed the doublure. This doublure, which may extend for some distance parallel to and beneath the dorsal surface of the shield, not infrequently shows the same type of surface ornament as the rest of the cephalon, but in some genera this condition is slightly modified by "terraced" lines running parallel with the borders. The ventral labrum, or hypostome, is attached to the doublure so as to lie beneath the front part of the glabella (or axial part of the cephalon) and is usually ornamented on its ventral surface in a manner similar to the doublure; the hypostome itself is, however, usually provided with a marginal dorsally reflexed doublure except along its anterior margin.

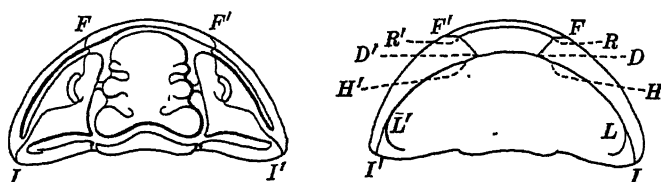


Fig. 1. The cephalic sutures in *Calymene blumenbachi* Auctorum (Middle Silurian, Dudley)—comparative interpretations. The hypostome is not drawn in its position in life, for the suture $H'D'DH$ should really lie more forward under the doublure and nearer the glabella. According to:

i. *Barrande*: $RFIL$ (facial suture) with RR' (rostral suture) and $R'F'I'L'$ (facial suture) together form *la grande suture*; RD , $R'D'$ (connective sutures) and $HDD'H'$ (hypostomal sutures) are two units of an origin independent from that of *la grande suture*.

ii. *Beecher*: $DRFIL$ bounds the pleural part of a primary segment; $DRR'D'$, the central part of the same segment; $HDD'H'$, is a primary segmental boundary; $I'F'R'RFI$, another primary segmental boundary.

iii. *Warburg*: $RFIL$, $R'F'I'L'$ are branches of one unit; RD , $D'R'$ together are another unit surrounding the rostrum which according to Kiaer is a primary segmental unit.

iv. *Henriksen*: $RFIL$ with RD together form one unit; $R'R$ is a relic of an older unit, $HDD'H'$ is a primary segmental boundary.

v. *Rud. Richter*: DR with $RFIL$ form one primary unit; RR' is a newer suture.

Typically the hypostome is separated anteriorly from the cephalic doublure by a transverse cleft (see Fig. 1, $H'H$) called the hypostomal suture (*suture hypostomale*, Barrande, 1852, p. 115),¹ but in many Cambrian species the place of this suture (usually present in all post-Cambrian and some Cambrian genera) is occupied not by an open suture but by an impressed line (Fig. 2b) and the hypostome and doublure there form one plate.

The lateral and anterior margins of the cephalon may be the locus of a somewhat similar open suture which enables the cephalic doublure to be completely detachable from the rest of the cephalon (*e.g.* in certain species of *Harpes* Goldfuss; for illustration see Richter, 1921, Pls. XVII, XVIII); or this suture may slightly

¹ In certain genera (*e.g.* *Iliaenus* Dalman, *Calymene* Brongniart) where the cephalon is domed, the antero-lateral cephalic doublure has posteriorly a second or dorsal flexure, and the hypostomal suture may lie dorsal to the main mass of the doublure.

transgress the dorsal surface to run inwards postero-laterally (Cryptolithidae; for illustration see Störmer, 1930); this is called the marginal suture (*Randnaht*, Beyrich, 1846, pp. 29, 32).

Of the cephalic sutures however, the best known and most easily seen is the facial suture (*sutura* or *linea facialis*, Dalman, 1827; 1828, p. 12), which is present in the majority of trilobite genera and is composed of two lateral branches (Fig. 1, *RFIL*, *R'F'I'L'*) starting symmetrically on the posterior or lateral doublure, crossing the border and taking a symmetrical course along the dorsal surface of the cephalon to bound the axial side of the visual surface of the paired eye,¹ continuing forwards either to unite anteriorly on the dorsal surface (Fig. 2*E*) or marginally (Fig. 2*F*). Sometimes these two branches meet the antero-lateral angles or anterior edge of the unpaired median rostral shield, or rostrum, which may be part of the doublure (Fig. 2*c*; Fig. 1, *R'RDD'*) or may be partly on the dorsal surface as in *Homalonotus* König (Fig. 2*D*), or *Encrinurus* Emmrich. The anterior rostral boundary suture is known as the rostral suture (*Schnautsennaht*, Burmeister, 1843, p. 25). The term "rostral" was also applied by Warburg (1925, p. 37) to the submarginal suture (Fig. 2*a*) found in certain of the Mesonacidae; this same suture was termed "marginal" by Swinnerton (1919, p. 106) and others, but Rud. Richter (1932*a*, p. 139) would prefer to have this mesonacid suture called "perrostral", and it seems best to adopt this name since the homologies with either a rostral or marginal suture, as defined above, have yet to be proved.

In certain forms (in the families Cyclopygidae, Phacopidae, Nileidae, Remopleuridae) the hypostomal and facial sutures are not united by open sutures (Fig. 2*f*), and in these trilobites the rostrum is not present as a separate plate bounded by sutures; in other words, the part of the cephalon anterior and ventral to the supposedly united facial sutures is directly continuous with the doublure (except for the hypostome which is separated by the hypostomal suture). In some other forms, junction between the facial and hypostomal sutures is effected by connective sutures (*sutures de jonction*, Barrande, 1852); when these occur as a symmetrical pair, they have been designated by Barrande "sutures jumelles" as in Calymenidae (Fig. 1, *DH*, *D'H'*); should there be, however, only a single axial suture as in Asaphidae (Fig. 2*e*) or in some forms of the Dikellocephalidae, Barrande's term median suture is used. In trilobites where the rostral suture occurs, or the facial sutures unite, on the dorsal surface, the connective or median sutures may be continued from the doublure on to the dorsal surface (Fig. 2*D*, *E*); or alternatively if the doublure is suppressed anteriorly because of the forward position of the hypostome, the connective sutures² may exist almost entirely on the dorsal surface, even transgressing on to the glabella as in *Encrinurus* Emmrich.

In species where the hypostomal suture is closed or absent and where the rostrum is ventrally fused to the hypostome, the connective sutures cross the doublure to

¹ A facial suture may exist where the compound eye is absent (Figs. 5, 6), but its course is then similar to that in the nearest relative of the eye-bearing trilobite.

² These encrinurid connective sutures have been called rostral sutures by Reed (1928, p. 70), a misleading use of a well-established term (see above).

terminate somewhere near the anterior limit of the true hypostome (*e.g.* some species of *Paradoxides* Brongniart, Fig. 2*b*, where the rostral suture is marginal).

All these open sutures have a constant position in an adult cephalon of a species, and, having no precise homologues among living arthropods, have long attracted comment. Three explanations of their functions have been suggested:

(1) Burmeister (1843, p. 26) supposed that they allowed slight movements of the skeleton when the animal enrolled and thus improved the protection of the appendages and soft parts; (2) Barrande (1852, p. 113) considered that the sutures had no other purpose than to facilitate ecdysis, and this explanation is still widely accepted; (3) Zittel (1875, p. 143), Brögger (1886), and Öpik (1929) argued with varying degrees of assurance that at least some of the sutures had a part in the trilobite's feeding mechanism. That the hypostomal suture functioned to this end is an opinion not difficult to accept, and in those forms, such as certain species of the Mesonacidae and Paradoxididae, where the hypostome is fused to the hypostome attachment or rostrum, conceivably the perrostral or rostral suture replaced the normal function of the hypostomal suture. With the present scanty morphological information available, it is, however, hazardous to affirm that a hinge movement at the hypostomal or rostral suture definitely produced a complementary movement along the remaining cephalic sutures, although such a movement is conceivable.

It cannot be denied that the presence of cephalic sutures facilitated ecdysis, but it is at least arguable that the sutures existed only for this purpose.

III. INTERRELATIONSHIPS OF THE CEPHALIC SUTURES

Barrande's comparative studies of these trilobite structures are classical even though every worker has not accepted his conclusion that the facial, rostral and marginal sutures are part of a single unit termed by him (1852) "la grande suture". This unit he claimed as of an origin completely independent from that of the connective sutures and of the hypostomal suture. That the marginal suture (as defined on p. 409) is a distinct unit from the facial suture is generally agreed by modern European writers, but the question of the united facial and rostral sutures being of an origin independent from that of the connective sutures is a matter of dispute. Kiaer (1916) and Warburg (1925) claimed the rostral suture as a possible phylogenetic remnant of the earlier mesonacid [per-] rostral suture (Fig. 2); if this be so, then the connective sutures are also looked upon by these authors as remnants of the same mesonacid suture. This view is thought by its exponents to give further support to Barrande's tenet that the paired connective sutures could be replaced by a median suture, and these later writers believed that this was effected in trilobite phylogeny by a reduction in area of the rostrum and the ultimate meeting of the connective sutures as a median suture which, they suggested, eventually disappeared. As yet, however, nothing approaching a phylogenetic suite has been produced to substantiate this hypothesis; furthermore there is some evidence in the Asaphidae, that the median suture is a secondary structure developed after the

rostrum had become fused into the doublure (Raymond, 1917, p. 202; Reed, 1931, p. 452).

Others, including among recent writers, Henriksen (1926) and Rud. Richter (1932), envisaged the connective sutures as direct forward and ventral continuations of the facial suture branches. Barrande (1852, p. 121) who had argued against this view, drew attention to the course of the sutures in species of *Goldius* de Koninck (= *Bronteus* Goldfuss) and *Iliaenus* Dalman, where the facial and connective suture branches do not unite to form a smooth curve or straight line; in these forms, however, he observed that a smooth curve connects the facial and rostral sutures and this, he thought, indicated intimate genetic association between these, rather than between the facial and connective sutures (see also Fig. 1, where *FRD* also does not produce an even curve). A reduction of the rostrum, as Kiaer and Warburg suggested, would perhaps explain this condition without invoking kindred genesis,

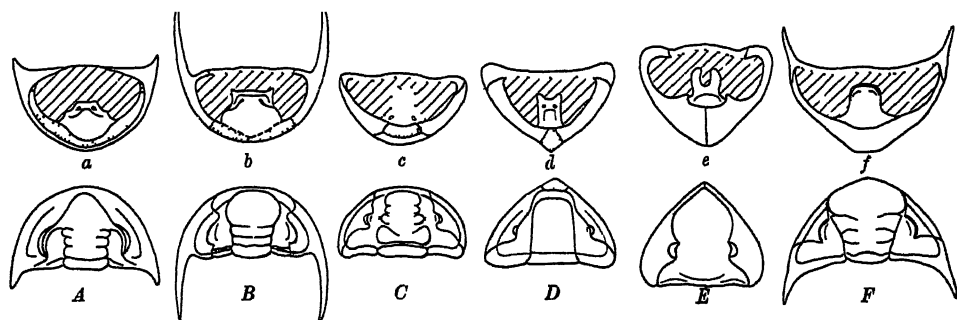


Fig. 2. Cephalons arranged as a morphological (but not phylogenetic) series illustrating the Kiaer-Warburg hypothesis of the reduction of the rostrum (modified from Rud. Richter, 1932, p. 843, Fig. 1). The rostrum is shown by stippled shading. A, a, *Kjerulfia lata* Kiaer; B, b, *Paradoxides bohemicus* Boeck; C, c, *Calymene blumenbachii* Auctorum; D, d, *Homalonotus laevigatus* Quenstedt; E, e, *Isotelus gigas* Auctorum; F, f, *Dalmanitina socialis* (Barrande).

if the rostrum was becoming diminished in such a manner as to modify the course of the facial sutures as little as possible.

Henriksen's interpretation of the connective sutures as being part of the facial sutures is complicated by his belief that these last were later phylogenetically than the perirostral suture, for a new factor is hereby introduced into the Kiaer hypothesis of the reduction of the mesonacid hypostome attachment or "rostrum". As Rud. Richter (1932a, p. 145) has stated, the supposed descendants of the Mesonacidae according to Henriksen, would retain as a separate plate, not the laterally reduced mesonacid rostrum, as Kiaer and Warburg believed, but only the central portion of this, for the side portions would presumably be separated from this by the superimposed facial sutures and would disappear, or be fused into the lateral cephalic doublure.

Rud. Richter (1932, p. 842) discussed some of the above interpretations and suggested that a true explanation will only emerge when more is known of Lower Cambrian trilobites other than the family Mesonacidae, which family he apparently regarded as specialised rather than primitive in its sutures. He wrote

of some unpublished observations of Resser's that in these (non-mesonacid) Lower Cambrian forms frequently there are a pair of long sutures passing across the dorsal cephalic surface and crossing directly on to the ventral doublure without the intervention of a cross-suture (rostral) uniting these. Richter assumed these sutures (Fig. 3 i) to be the original sutural element of which part (Fig. 3 i, ESC , $E'S'C'$) will eventually become a dorsal arch (*dorsalbogen*) and the rest (CF_1 , $C'F_1'$) the connective sutures. He called these two long branches ($ESCF$, $E'S'C'F_1'$) the facial

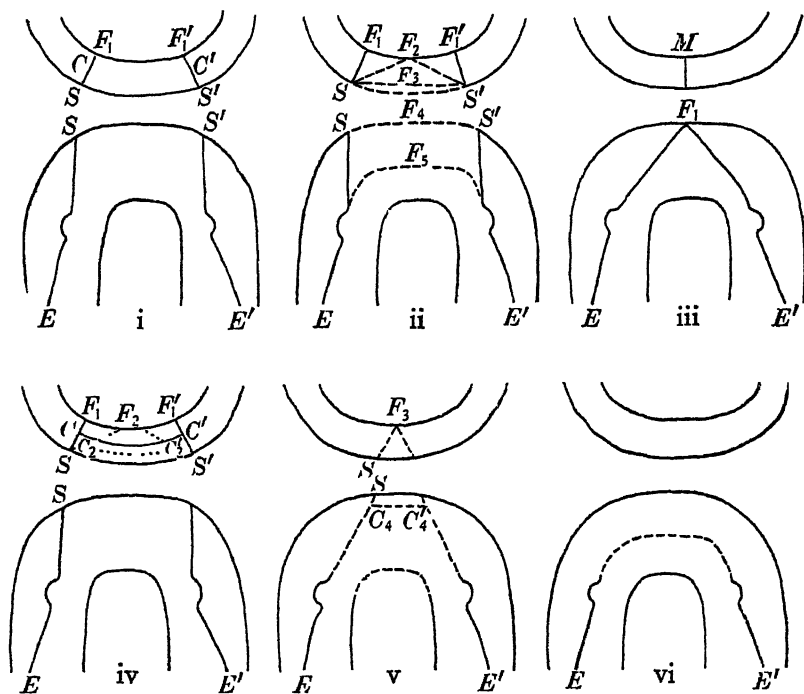


Fig. 3. Diagram illustrating trends as suggested by Rud. Richter in evolution of some cephalic sutures (modified from Richter, 1932, p. 843, Fig. 1).

- i. Supposed original cephalic sutures, $ESCF_1$, $E'S'C'F_1'$.
- ii. Suture ESF_1 evolving to take up ultimate position EF_5 .
- iii. Suture EF_1E' connected to margin of doublure by suture F_1M which may eventually disappear.
- iv-v. Sutures $ESCF_1$, $E'S'C'F_1'$ connected by the new element CC' evolving to C_2C_2' and C_4C_4' eventually to reach stage vi, or, by a different path, stage iii.
- vi. Ultimate stage reached by ii, iii, iv, v.

sutures and considered that the tendency would be for the two sutures to unite anteriorly (Fig. 3 ii); this junction could take place, as evolution proceeded, at the margin of the doublure (Fig. 3 ii, $ESF_2S'E'$), on the doublure itself ($ESF_3S'E'$), at the frontal margin ($ESF_4S'E'$) or on the dorsal surface (Fig. 3 vi). Should the junction not be effected at the doublure margin, then, suggested Richter, such junction might be effected with this margin by a median suture (Fig. 3 iii and Asaphidae, Fig. 2e) or not at all (Fig. 3 ii, F_3 , F_4 , F_5 ; Fig. 3 vi, Phacopidae, Fig. 2f).

Alternatively if the two branches remained at the doublure margin a supplementary cross-suture (Fig. 3 iv, CC') might come into being—this is the rostral suture (as defined by Burmeister) and the rostral plate or rostrum would then be a secondary structure and not primary as Kiaer¹ and Warburg have claimed. With the reduction of the rostrum and the rostral suture, a median suture (Fig. 3 iii) might arise and finally the condition shown in Fig. 3 vi. The rostrum might also, as Warburg (1925) had previously suggested, become fused into the doublure and the rostral suture might survive as the linking suture of the decapitated branches of the facial suture (Fig. 3 v, C_4C_4' , Fig. 3 vi).

This hypothesis of Richter's is very speculative, for he gave no tangible evidence which may be termed either phylogenetic or ontogenetic in confirmation of any step in the thesis; furthermore no Lower Cambrian genus is mentioned by name as showing the remarkable "facial sutures" without an associated cross-suture (Fig. 3 i). It would be interesting to learn whether or not these particular Lower Cambrian genera are provided with hypostomal sutures.

The cephalic doublures of many trilobite species are as yet undescribed, and until more facts are available it is perhaps better to refer to the facial and connective sutures according to the definitions given by Barrande.

Of the known individual evolutionary movements of particular sutures the undisputed facts are few. The facial sutures are believed, within the limits of particular families, to be capable of a change of locus (Phacopidae, Fig. 5; Proetidae, Fig. 6); likewise are the connective sutures in other families (Encrinuridae, if the assumption is accepted that *Cybele* and *Encrinurus* come from the same ancestral stock). There is also (ontogenetic) evidence that within the life of an individual the facial sutures may in some cases modify their position considerably during growth (Triarthridae, Solenopleuridae, see p. 427 and Fig. 9). It may be further noted that each of the three types of typical suture, namely, facial, connective and hypostomal, individually may be present or absent within the limits of certain families, or even genera. Members of the Odontopleuridae are known with an open or closed facial suture, and in such species as *Acidaspis* (*Ceratocephala*) *verneuili* (Barrande) (Fig. 4b) and *A. (C.) vesiculosa* (Beyrich) the closing of the suture is with fair probability considered to be a secondary feature. Though the median (connective) suture is apparently open in some species of *Dikellocephalus* Owen, e.g. *D. raaschi* Ulrich & Resser (Ulrich & Resser, 1930, Pl. X, fig. 2), this suture is closed in a group of species of which *D. subplanus* Ulrich & Resser (*op. cit.* Pl. XIV, fig. 4) may be named as an example, but here again the fusion is likely to have been secondarily effected.

In the Mesonacidae, the hypostomal suture may be open or closed, but in this family (Kiaer, 1916, pp. 85, 86) there is so much "specialisation crossing" (in the sense that this expression is used by Dollo and Abel) that it is difficult to decide which genera are the most primitive; and it cannot convincingly be claimed that the closing of the hypostomal suture is secondary rather than primary despite the

¹ Kiaer thought the rostral suture and its supposed homologue, the perirostral suture, to be a primary segmental boundary.

fact that in the geologically later though not necessarily derivative family, Paradoxididae, the fusion, might be held to post-date an original suture, since some early species appear to have such a suture. In the present state of our knowledge concerning many Lower Cambrian genera, and particularly the ventral surfaces of these, it is not yet possible to prove whether or not the trilobites were primitively provided with a hypostomal suture, or even any suture. If, however, the Mesonacidae can be held to be primitive in respect to the absence of a hypostomal suture,

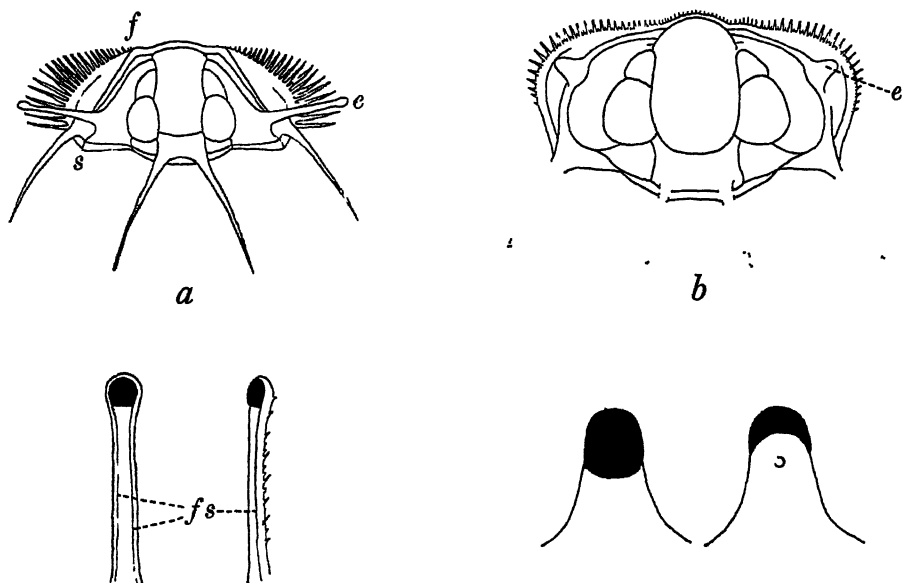


Fig. 4. Cephalons of trilobites belonging to the same family in which the facial suture (*fs*) is open or closed despite the presence in each case of lens-bearing compound eyes (*e*). *a*, *Acidaspis* (*Odontopleura*) *mira* Barrande, $\times 3/2$; also enlargement of the elevated eye of which visual surface is indicated by heavy shading, $\times 7$. *b*, *Acidaspis* (*Ceratocephala*) *verneuili* (Barrande), $\times 1$; also enlargement of the eye, visual surface shaded as in Fig. 4*a*, $\times 7$. Drawn from figures in Barrande (1852).

it may well be that the perrostral suture there present had some function in the bionomics of the animal which was subsequently taken over by the hypostomal suture (see p. 410).

IV. CLASSIFICATION BY CEPHALIC SUTURES

Barrande (1852), after a comprehensive study of the trilobite genera known to him, looked upon the cephalic sutures and their combinations as having little more than generic value in classification; he evolved a major grouping of trilobite families based on the configuration of the thoracic pleurae, a scheme which has now been abandoned because within the same family, Cheiruridae, the two types of pleurae may occur. Barrande thought, however, that the course of a part of the facial sutures might establish links of genera into families.

Despite these views, Salter (1864, pp. 1-2) in his masterly but unfinished "Monograph of British trilobites", found favour in Emmrich's conclusion that

the facial suture, especially the course of its posterior branch, had much stronger classificatory importance than Barrande would allow, and he established four groups of trilobites: "(1) Without eyes or facial sutures (Agnostini). (2) Facial sutures obscure or submarginal or none. Eyes often absent (Ampycini). (3) Facial suture ending on the posterior margin. Eyes (usually) moderately developed, smooth (Asaphini) [Fig. 2E]. (4) Facial suture ending on the external [lateral] margin. Eyes well developed, usually faceted externally (Phacopini) [Fig. 2F]." Salter also believed that two main trends of segment distribution existed in trilobite evolution.

This sutural grouping was modified in details by Beecher (1897), who claimed that facial sutures were (in the sense used by Salter) homologous for all trilobites, and Salter's groups Agnostini and Ampycini were combined together into an order, Hypoparia, augmented by the family Harpidae [Harpedidae *sic*] from the group Asaphini. Beecher stated that the forms he had thus compounded shared the common character of well-developed, continuous ventral free cheeks (*i.e.* parts of the cephalon outside the facial sutures). Salter's group Phacopini, Beecher renamed as the order Proparia, defining it as one in which the free cheeks occur in front of the genal (postero-lateral cephalic) angle and he added to the group, the family Calymenidae included by Salter in the group Asaphini. For the remnants of the Asaphini, Beecher constituted a new order Opisthoparia "where the free cheeks include the genal angle" (Beecher, 1897, pp. 100-1).

Salter had considered the Agnostini "to be the lowest and most primitive group", an opinion which Beecher accepted and to which he adduced support from his own and others' previously published comparative studies of early trilobite developmental stages; he had in fact maintained that the Hypoparia, most closely of all three orders, resembled the "primitive" trilobite larva which he visualised as an archetype. So strongly did these larval studies influence him that he claimed his tripartite grouping as "an outline of a natural classification".

As a caveat Beecher (1897, p. 97) remarked that when formulating a classification in which ontogeny plays a great part, "it is of the greatest importance also to study the ontogeny of primitive and non-specialized species because without very complete paleontological evidence, the development of a much later derived form may be so involved with larval adaptations and accelerated characters as to be misleading". His evaluation of the primitive in trilobites was, however, unfortunately based on studies of early stages, not of Lower Cambrian species but of Middle Cambrian forms belonging to the genera *Solenopleura*, *Liostracus* and *Elrathia* [*olim Ptychoparia*]. From these species he deduced (Beecher, 1897, p. 100) that "any trilobite with a large elongate cephalon, eyes rudimentary or absent, free cheeks ventral or marginal, and glabella long, cylindrical, and with five annulations, would naturally be placed near the beginning of any genetic series or as belonging to a primitive stock". Thus he placed the Hypoparia as the most primitive of his three groups and the apparently blind family Conocoryphidae as the radicle of his group Opisthoparia, whilst the family Encrinuridae, which contains some blind forms, was relegated to the base of his group Proparia. The family which

dominated Lower Cambrian seas over Europe and North America, namely the Mesonacidae, he declined to consider as primitive, since the earliest known developmental stage or "protaspis" did not conform to what he had thus adduced as the basic form.

That Beecher's classification was "natural" was quickly challenged by Pompeckj (1898, p. 188 footnote) on the general ground that it was merely a mode of expressing the equal stages reached along different lineages by a single character, viz. the free cheeks.

Another objection could be raised, namely that the scheme has the same inherent objection as had Barrande's, for in the family Calymenidae, certain species of the genus *Calymene* have proparian free cheeks, whilst in the genus *Pharostoma* Corda, the free cheeks are opisthoparian (p. 428), and judging from figures given by Barrande similar examples may be chosen from the families Lichidae, Encrinuridae and Cheiruridae. This last proparian family contains not only the opisthoparian genus *Placoparia* Corda but also *Crotalurus* Volborth (F. Schmidt, 1907, p. 16, pl. I, fig. 11).

However, Beecher appeared to lay himself most seriously open to criticism by his assumption that some of the cephalic sutures were occupying the positions of primary segmental boundaries, and this led him to establish homology between the marginal sutures of the Hypoparia and the facial sutures of the remaining "orders".

As will be shown later, there is some evidence that the post-ocular portions of proparian and opisthoparian sutures are homologous, but the evidence that even these are necessarily relics of primary segmental boundaries is very hypothetical.

(i) *The present position of Beecher's order Hypoparia*

The integrity of this order was soon called to question, for Pompeckj (1898, p. 188 footnote; 1903, p. 502) strongly doubted whether there was enough affinity between the genera *Agnostus* Brongniart and *Harpes* Goldfuss to warrant that they should be classed together in the same "natural order". Later Lindström (1901), Holm and also Jaekel (1909) doubted the existence of a marginal suture in the *Agnostus* cephalon, a doubt upheld to the present day. Jaekel (1909) went further and concluded that the agnostids and eodiscids were not primitive but highly specialised forms, and that this specialisation was so marked as to warrant these forms being placed as a subdivision of the trilobites equal in grade to one comprising the rest of the group. Howell (1935) recognises the force of Jaekel's argument and classes the agnostids (*sensu stricto*) as an independent order. It was, however, the work of Lake (1907, pp. 44, 45) which first produced a challenge to the homology of the hypoparian marginal suture with the true facial suture. Lake suggested that the Tremadoc (late Cambrian) genus *Orometopus* Angelin might be a facial suture-bearing ancestor to the Ordovician hypoparian genera *Trinucleus* Murchison and *Ampyx* Dalman, and this Tremadoc genus, in all known species, is provided with compound eyes.

Beecher had modified an hypothesis of Bernard's, namely that the Trilobitae¹

¹ Rud. & E. Richter (1925, p. 239) stated that the group name should really be so written since it is etymologically more correct than Trilobita.

and Crustacea were derived from polychaete worms by the longitudinal bending of the anterior segment or prostomium, to arrive at the conclusion (Beecher, 1897, pp. 95, 96) that the trilobite hypostome was the original first segment, and that the eye-bearing free cheeks with the rostrum were the original second segment. These two segments, he stated, retained signs of their original individuality by being bounded transversely by sutures, and he claimed that in the Hypoparia the second segment remained "where it was mechanically placed" and thus was primitive. As evolution proceeded, he maintained (Beecher, 1895, pp. 177-8) that trilobite eyes "migrated from the ventral side [*i.e.* from the prostomium], first forward to the margin and then backward over the cephalon to their adult position.... Therefore the most primitive larvae should present no evidence of eyes on the dorsal shield, and naturally there would be no free-cheeks visible." Beecher offered no evidence of ventral eyes having been seen in larval or adult trilobites, and his deductions were accompanied by a regrettable inconsistency for the Harpidae was stated (Beecher, 1897, p. 186) to be "the only family known in which functional visual spots, or ocelli, are situated on the fixed cheeks. The young *Trinucleus* has similar [and similarly situated] eye spots or ocelli."

These latter statements, taken in conjunction with that in which the free cheek (separated from the fixed cheek by the facial suture which was on slender evidence assumed by Beecher to be a primitive segmental boundary) was claimed to be the eye-bearing segment, are difficult to correlate with Beecher's "theory" of phylogeny, particularly when he dogmatically asserted (1897, p. 95) that "the structural position [of the eyes in relation to segmentation] in the trilobites is invariable".¹ It is not surprising, therefore, that Lake should doubt the perfect homology of the *Orometopus* free cheeks with the ventral "free cheek" of *Trinucleus*.

Beecher's disciple Raymond, however, in 1913 retained the name Hypoparia for an order comprising the families Agnostidae, Eodiscidae, Shumardiidae, Harpidae, Trinucleidae [= Cryptolithidae] and Raphiophoridae. Rud. Richter (1914) then announced that the eye-spots of the hypoparian genus *Harpes* Goldfuss were vestiges of once well-developed compound eyes, and suggested that the marginal suture of *Harpes* could not be correctly considered homologous with the facial sutures of opisthoparian and proparian trilobites. It should perhaps be remarked here that Richter believed that facial sutures could not exist in a trilobite having compound eyes unless those sutures bound the inner margins of the eyes in part of their courses.

The publication by Walcott (1916a, p. 407) of a description of the *Eodiscus*-like proparian genus *Pagetia*, found in Middle Cambrian rocks of British Columbia and Idaho, was made an opportunity by him to transfer the hypoparian family Eodiscidae to the Proparia. With these opinions collected before him, Swinnerton (1915, 1919) advocated the abolition of the order Hypoparia; and the component families, which he regarded as far as their "facial sutures" were concerned as degenerate members of the Pro- and Opisthoparia, he distributed as best he could

¹ In this connection Swinnerton (1919, p. 109) has concurred, stating "the segment which bears the eye is the same for all trilobites, so that from this standpoint the order is monophyletic".

among the two remaining orders of the Beecher classification. He erected (1915, p. 493) a group, however, to form for primitive "Trilobites and Trilobite-like organisms in which the absence of facial sutures is primary the Order Protoparia".

On *prima facie* grounds, it would perhaps appear more reasonable to suppose that facial sutures, rather than dorsally placed compound eyes, were evolved within the class Trilobitae, but the value of Swinnerton's new group has been questioned by Warburg (1925, pp. 54, 78-9), Raw (1925, p. 310) and more fully by Richter (1932, p. 856), because its suggested members include forms which are not trilobites nor necessarily ancestral to trilobites, and also because the Middle Cambrian genus *Nathorstia* Walcott, the only trilobite accepted as a member by Swinnerton, is now thought to be merely a "butter-crab" of the normally opisthoparian suture-bearing species, *Neolenus serratus* Röminger. It would seem, therefore, that until *Marrella* Walcott, and its relatives, can be convincingly shown to be members of the trilobite ancestral line, as Swinnerton believed, the order Protoparia has only an hypothetical significance and should be abandoned. Henriksen's (1928) researches have shown that *Marrella* may be considered as a true branchiopod crustacean, probably near to the Notostraca.

Before the publication of Swinnerton's important papers the natural validity of the order Hypoparia had been questioned in general terms amongst others by Gürich (1907), Lake (1907), Jaekel (1909), Woods (1909) and Pompeckj (1912). Swinnerton's criticisms have on the whole been favourably received by Rud. Richter (1921, p. 198) Ulrich (1922), Raw (1925), and Warburg (1925). Raymond (1917, p. 205), on the contrary could not accept the order as comprising degenerate members of the other two groups. He readily accepted *Pagetia* Walcott as an Eodiscid but remarked (1917, pp. 199-200) that since the genus was described from Middle Cambrian rocks and that *Eodiscus* Matthew already existed in Lower Cambrian times, he thought it likely that in *Eodiscus* the eyes were rudimentary rather than vestigial. If the two species figured by Cobbold (1931) from the Lower Cambrian rocks of Shropshire have been correctly interpreted as proparian and are really Eodiscidae, the force of Raymond's arguments is diminished.

An immature cephalon belonging to one of these *Pagetia* species (Cobbold 1931, Pl. 31, fig. 46) undoubtedly shows proparian sutures, but, of itself, as an immature specimen, this is not sufficient evidence of the proparian condition of the adult (see p. 427). The adult, moreover, shows characters which make the generic reference to *Pagetia* doubtful.

The Danish workers Henriksen (1926, p. 29) and Poulsen (1927, p. 311), whilst agreeing with many modern workers that the facial and marginal (*sensu lato*) sutures are not perfectly homologous, concluded as did Swinnerton that the Mesonacidae primitively lacked facial sutures, but Poulsen retained the name Hypoparia¹

¹ In 1927 (p. 315) Poulsen proposed the terms Integricephalida and Suturecephalida for the two divisions of Trilobitae without and with facial sutures respectively; the Mesonacidae constituted a third division termed Mesonacida of equal ordinal rank to these. After objections had been raised to the first two names on the grounds that they were etymologically hybrid and cacophonous, in 1932 (p. 48) he replaced the term Suturecephalida with Epiparia and in 1934 (p. 20) he revived the term Hypoparia for Integricephalida.

for an order comprising the families Agnostidae, Eodiscidae (excluding *Pagetia* Walcott), Conocoryphidae, Trinucleidae and Harpidae. He argued that though these trilobites may have reduced eyes or even be secondarily blind, they are derived from early members of the mesonacid stock because they retain the primary "marginal" [=perrostral] suture, which, he stated, existed before the facial suture in trilobite phylogeny. That the family Conocoryphidae should be included in the order Hypoparia was an unexpected event; Beecher thought this family to be primitively opisthoparian, as did Swinnerton, but Warburg (1925, pp. 45, 59) and Raw (1925, p. 261) had deduced that the members of the Conocoryphidae are really secondarily blind Ptychopariidae and therefore degenerate Opisthoparia.

Though Poulsen's (1927, p. 315) assertion may be correct that Barrande erred in attributing connective sutures to *Conocoryphe sulzeri* Schlotheim, for the evidence of the existence of these is far from conclusive, his argument (1927, p. 313) surely is unsound that because the conocoryphid suture is "outside the rim" of the cephalon and because "trilobites with a real facial suture show the reverse" therefore the conocoryphid suture is a marginal and not a facial suture, and hence the family should be placed in the Hypoparia. The Upper Devonian subgenus of *Phacops* called *Dianops* R. & E. Richter shows a similar suture which never leaves the region outside the marginal furrow of the cephalon and this suture has been shown by Rud. & E. Richter (1926, pp. 9, 184-90, also R. Richter, 1932, p. 845, fig. 3) to be a true facial suture which has migrated marginally with the loss of the compound eye (see also Fig. 5); it follows, therefore, that Poulsen's premise about the true facial suture always being completely on the inner side of the "rim" is untenable. It is likely that the Conocoryphidae represent a polyphyletic assemblage of short-lived secondarily blind forms in which, as an accompaniment to blindness, the facial suture has migrated marginally as in the Phacopidae; some of the Conocoryphidae may have originated as Ptychopariidae (Rud. Richter, 1932a, p. 182).

It has already been mentioned (p. 409) that the perrostral suture of the Mesonacidae is not necessarily a structure homologous with the marginal suture of the Cryptolithidae (Trinucleidae) and Harpidae.

With the assumption that the paired trilobite eye is homologous throughout the class, Richter (1921, pp. 200, 203) adopted Reed's (1916, p. 175) conclusion concerning the Trinucleidae and stated that in both this family and the Harpidae the marginal suture is not a facial suture but a new development consequent upon the loss of the facial suture and the formation of the cephalic fringe. If this view is correct, there would remain no justification for the retention of the Hypoparia as a natural group of primitively blind and marginally sutured trilobites, since of the remaining families, no cephalic sutures are positively known, marginal or otherwise, in the Agnostidae and Shumardiidae, and *Eodiscus* with its sutureless relatives are thought to be degenerate proparians as far as sutures are concerned. The dorsal suture-bearing genus *Ampyx* Dalman (and other Raphiophoridae) is, except by Raymond and his followers, now considered to have an opisthoparian suture in

much the same manner as the apparently blind suture-bearing species of *Illaenus* Dalman (*Illaenidae*), *Drevermannia* Rud. Richter, *Typhloproetus* Rud. Richter and *Pteroparia* Rud. Richter (*Proetidae*) are held to be opisthoparian.

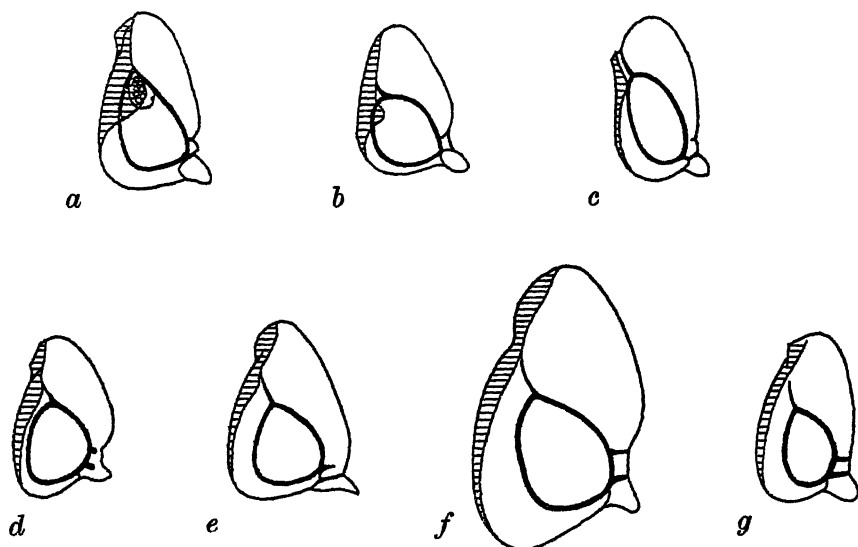


Fig. 5. Profiles of cephalons arranged in two (possibly connected) phylogenetic series (described by Rud. & E. Richter, 1926, p. 9), illustrating the lateral migration of the compound eyes, their disappearance and the progressive attainment by the facial suture of an unindented curve and a marginal course in Upper Devonian Phacopidae. One series contains the forms figured as *a-c*, the other *d-g*. The free cheeks are indicated by horizontal line shading. The zonal divisions are those of the German sequence. *a*, *Phacops* (*Cryphops*) *cryptophthalmus* (Emmrich), Zone II ?, $\times 4$; *b*, *Phacops* (*Trimeroccephalus*) *mastophthalmus* (Reinh. Richter), Zone II, $\times 3$; *c*, *Phacops* (*Trimeroccephalus*) *caecus* (Gürich), Zone II, $\times 4$; *d*, *Phacops* (*Dianops*) *typhlops* (Gürich), Zone IV or V, $\times 3$; *e*, *Phacops* (*Dianops*) *limbatus* (Reinh. Richter), Zone V+VI, $\times 3$; *f*, *Phacops* (*Dianops*) *griffithides* (Rud. & E. Richter), Zone V+VI, $\times 4$; *g*, *Phacops* (*Dianops*) *anophthalmus* (Frech), Zone VI ?, $\times 4$. Illustrations redrawn from Rud. & E. Richter (1926, Pls. 9, 10, 11).

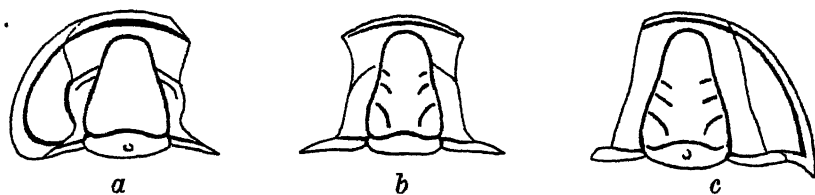


Fig. 6. Cephalons arranged in a morphological but not necessarily phylogenetic series illustrating the apparent loss of eyes and the subsequent straightening of the facial sutures in German Upper Devonian Proetidae. *a*, *Drevermannia palpebralis* Rud. & E. Richter, Zone I, $\times 5$; *b*, *Drevermannia adorfensis* Rud. Richter, Zone I, $\times 7$; *c*, *Drevermannia schmidtii* Rud. Richter, Zone V β , $\times 5$. Illustration redrawn from Rud. & E. Richter (1926, Pls. 5, 6 and Text-fig. 12).

Despite the fact that phylogenetic lineages have not yet been suggested to include any of the blind but facial suture-bearing species of *Illaenus* (e.g. *I. zeidleri* Barrande, *I. angelini* Holm¹), where the sutures take an abnormal course near to

¹ A fuller list of these species is recorded by Reed (1898, p. 500).

the margins of the cephalon, it is very probable that the species represent stages parallel to some of those described by Rud. & E. Richter (1926) in the Phacopidae.

In this last family the diminution of the number of lenses in the compound eye is accompanied by a migration of the eye and the facial suture towards the cephalic margin, culminating in apparently blind forms with a supra-marginal or marginal unindented facial suture (Fig. 5). The apparent loss of eyes in the proetid genera is not accompanied by such a pronounced centrifugal migration of the facial suture, though a straightening of the suture is observable.

With the elimination of the heterogeneously constituted order Hypoparia from its status as the most primitive of the "natural" groups of trilobites, it remains to consider the group which was thought to be the most primitive by Poulsen (1927), namely the "order" Mesonacida.

(ii) *The status of the Mesonacidae in classification*

Swinerton considered this family to form the earliest offshoot from his not generally accepted order Protoparia (see p. 418); he believed that in this family facial sutures were coming into being, an opinion shared by Poulsen (1927) on slightly different evidence. Whereas Poulsen stated that all trilobites were derived from the Mesonacidae, which family he raised directly to the rank of an order, Swinerton was content to derive from this, certain of the opisthoparian families which he united together with their supposedly ancestral family as a suborder Mesonacida; the remaining trilobite families Swinerton envisaged as having evolved along other paths from the Protoparia.

That the Mesonacidae are primitively without facial sutures is an opinion by no means universally accepted; it has been claimed by many workers that sutures exist in this group in a state of secondary fusion. Raw (1925, 1927), because of the supposed fused course of these sutures and the distribution of cephalic spines, would make the family the most specialised of all trilobites.

It has frequently been stated that the raised line or ridge (Fig. 7, *CI*), running from the back end of the eye towards but not necessarily touching the posterior margin of the cephalon, is the fused, post-ocular branch of the facial suture. It is, however, to be noticed that in those members of the Odontopleuridae (see Fig. 4), Otarionidae (*Brachymetopus* McCoy and *Brachymetopina* Reed), and Phacopidae where facial sutures are thought to be vestigial, the locus of the fused sutures is never represented by a raised line or ridge, and the same absence of elevation is known with other supposedly vestigial cephalic sutures; a groove is more usual. It is here considered that the post-ocular ridge in Mesonacidae (Fig. 7, *CI*) may not be a fused suture but rather, as Lindström (1901, pp. 15, 16) suggested, a vestige or remnant of the post-ocular part of the "larval ridge" (for definition see Warburg, 1925, p. 21) seen in early stages of mesonacid life histories (Fig. 8*a*), where the ridge occurs as an arcuate structure running outwards from the front part of the glabella. In certain stratigraphically later trilobites, where the lateral extension of the larval ridge becomes in part the adult palpebral lobe, this palpebral lobe has a

thin ridge or eye-line (Fig. 9c) connecting it to the glabella; developmental history shows that in certain families (Ptychopariidae, Olenidae) this eye-line is an attenuated anterior part of the larval ridge.

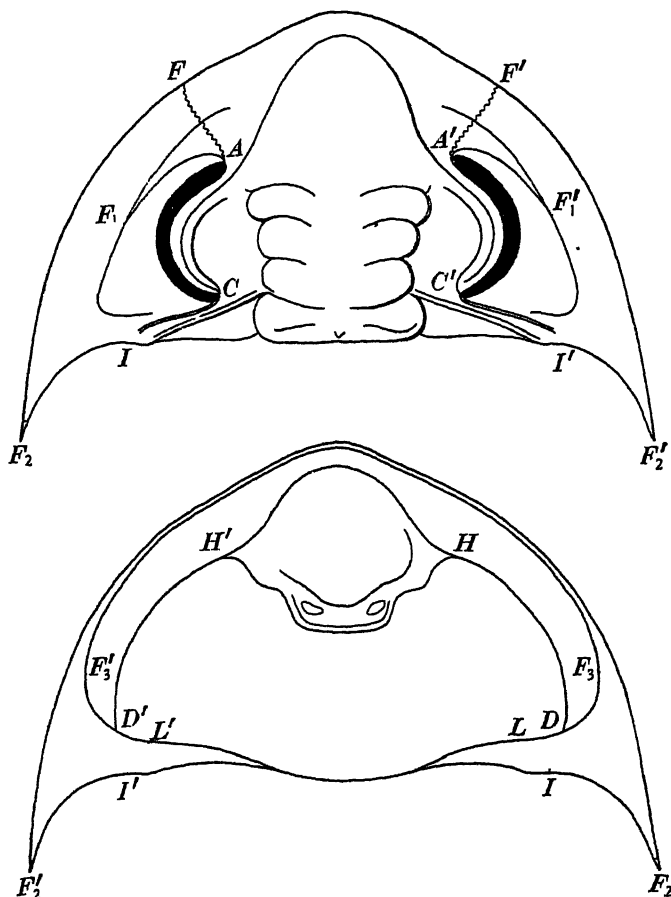


Fig. 7. Diagram of the dorsal and ventral side of the cephalon of *Kjerulfia lata* Kiaer (Lower Cambrian, Tømten, Norway) to illustrate comparative interpretations of the so-called sutures in Mesonacidae (based on illustrations from Kiaer (1916) with the addition of the lines FA , $F'A'$ from Poulsen (1927), $\times 2/3$). F_1A , $F_1'A'$ are slight furrows; IC , $I'C'$ are raised lines; FA , $F'A'$ are cracks (diagrammatically shown) in the shield. The only open sutures are $D'F_3'F_3D$, and $H'H$. According to:

i. *Moberg and Kiaer*: F_1ACI is a fused facial suture (of which ACI is posterior limit of primary eye segment); $D'F_3'F_3D$ is rostral [=perrostral] suture; $H'H$ is 'fused' hypostomal suture. Kiaer is not clear in his description as to whether the hypostomal suture is closed or open (see Kiaer, 1916, pp. 76, 77). Dr C. E. Resser, having examined the material, assures me that in *Kjerulfia lata* the hypostomal suture is open, but closed in *Holmia kjerulfi* (Linnarsson).

ii. *Swinerton*: F_1ACI is a rudimentary (incipient) facial suture; $D'F_3'F_3D$ is marginal [perrostral] suture.

iii. *Poulsen*: $FACI$ is an incipient facial suture (FA a line of weakness, CI , posterior limit of eye segment); $D'F_3'F_3D$ is marginal [perrostral] suture.

iv. *Raw*: $DF_3F_2F_1ACIL$ is fused facial suture of which lines $F_3F_2F_1$ and IL are admittedly hypothetical (F_3D is connective suture or a ventral extension of facial suture); $F_3'F_3$ is antero-marginal suture.

In mesonacid ontogeny the larval ridge, in the early stages of some species, would appear to reach the posterior margin of the shield, and as development proceeds, judging from the illustrations given by Ford and Walcott for *Elliptocephala asaphoides* Emmons, the eye and palpebral lobe develop from this ridge leaving a thin fillet-like elevation continuing to or towards the posterior margin

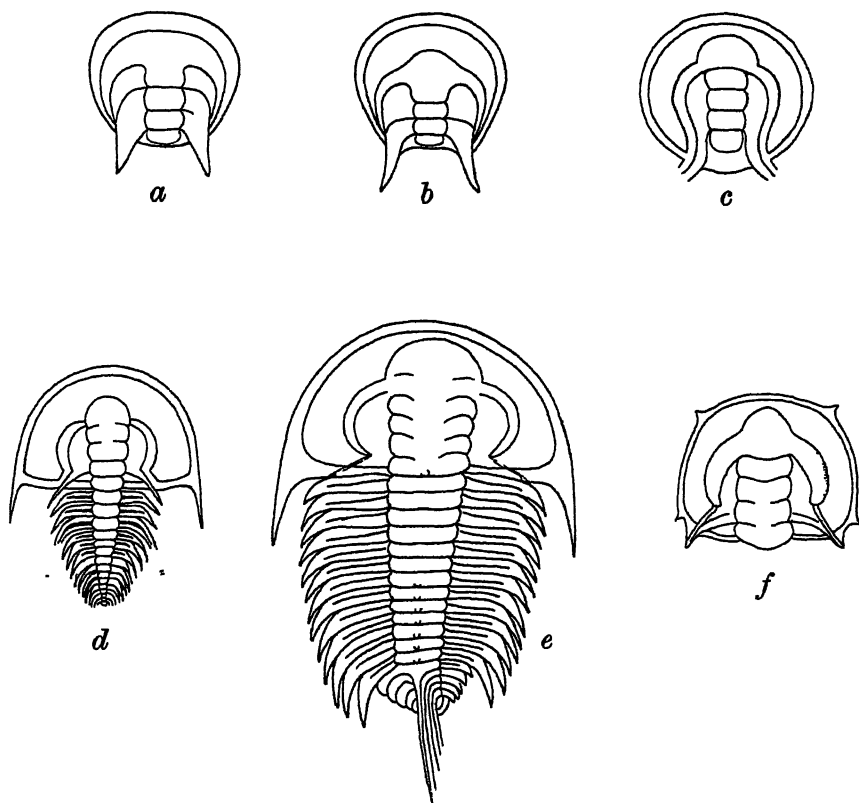


Fig. 8 *a-e*. Some developmental stages of the mesonacid *Elliptocephala asaphoides* Emmons. Lower Cambrian, New York State. Redrawn from Walcott & Ford. *a*, protaspis, earliest described stage, $\times 22$ (Walcott, 1886, Pl. XVII, fig. 5); *b*, slightly later, $\times 10$ (Walcott, 1886, Pl. XVII, fig. 6); *c*, later stage, $\times 10$ (Ford, 1877, Pl. facing p. 265, fig. 1); *d*, nepionic or early meraspid stage, $\times 4$ (Ford, 1881, Text-fig. 2, p. 251); *e*, adult as restored by Walcott, $\times 5/12$ (Walcott, 1910, Pl. XXIV, fig. 1). The interpretation given by Warburg (1925, p. 21) that the most posterior segment seen on the axis in Figs. *a-c* belongs to the telson or anal somite is accepted here. *f*. A small cephalon of "*Olenellus*" *gilberti* Meek (Lower Cambrian, Alberta) showing three pairs of marginal spines, including the intergenal spine arising from pre-occipital lobe of glabella, post-ocular ridge and part of visual surface of right eye, $\times 12.5$ (Walcott, 1910, Pl. XLIII, fig. 6).

(Fig. 8*d, e*), and the suggestion is made that the post-ocular ridge of the meraspid (for definition see Raw, 1925) and adult mesonacid is a structure somewhat homologous with the (pre-ocular) eye-line of certain other trilobites. If the mesonacids gave rise to facial suture-bearing trilobites, as Swinnerton and Poulsen would urge, it is possible that the post-ocular branch of the new facial suture might originate on one of the boundaries of this post-ocular ridge—the evidence of such

evolution however, cannot yet be considered to be conclusive. Whereas Raymond (1917, p. 208) suggested, and Poulsen (1927) believed, that the pre-ocular branch of the facial suture originated as a crack in the dorsal shield of the cephalon in a position roughly determined by the contour of the underlying hypostome, Warburg (1925) and Raw (1925) followed Moberg (1899) and Kiaer (1916) in thinking the locus of the pre-ocular branch (secondarily fused in Mesonacidae) to be determined by a thin upraised line (*Kjerulfia lundgreni* Moberg sp., 1899) or furrow (*Kjerulfia lata* Kiaer, 1916) running back and outwards from the anterior end of the eye. This line has, however, never been observed to cross the marginal furrow in any mesonacid species.

It should be remarked that in deference to the theory that evolution is irreversible, both Kiaer and Warburg have affirmed that if the mesonacid sutures really exist in a state of secondary fusion, the family could not have given rise to facial suture-bearing descendants, so that any resemblances which exist between Mesonacidae and other families (e.g. Paradoxididae, Redlichiidae, Zacanthoididae) must have arisen by convergent evolution resulting from common descent.

Raw (1925, 1927) also has taken this view; his opinion of the highly specialised nature of the Mesonacidae, built on a belief in the fundamental importance of certain spines in trilobite phylogeny, leaves the family placed at an acme of trilobite evolution along the path postulated by him, although he (1927, p. 22) also suggested that early mesonacids may have evolved into xiphosurids.

Raw's "Theory of Trilobite Phylogeny" (Richter, 1932a, p. 181) has its basis historically, in statements published by Walcott (1910, pp. 237-8), who wrote that the three pairs of spines observed in certain (immature) mesonacid cephalons (Fig. 8f) undoubtedly represent the pleural ends of segments that have fused together and greatly modified in the process". Walcott implied that the most posterior, or intergenal, pair of these spines persisted in certain opisthoparian genera (e.g. *Hydrocephalus* Barrande now considered by Raymond, Chouff and others to be a young *Paradoxides*) and occurred atavistically in certain species of *Goldius* de Koninck [= *Bronteus*], also that they formed the genal (fixed cheek) spines in all Proparia and in certain species of *Agnostus*. The mesonacid adult genal spines, or the middle pair of the three seen in some immature specimens, he regarded as the outer terminations of the ocular segment; these he thought persisted as the opisthoparian (free) cheek spines, and the anterior pair he suggested might probably belong to a pre-ocular segment of the cephalon. The mesonacid genus *Olenelloides* Peach is the only "adult" observed to possess all three pairs of spines, and even this form is doubted by some (Beecher, Warburg) as being adult; an early stage of "*Olenellus*" *gilberti* Meek has, however, been described as possessing the full spinose complement (Fig. 8f), as also, but less convincingly, an early stage of *Paedeumias transitans* Walcott.

Raw amplified this thesis and deduced that some of these various spines may coalesce in the course of development, but he stated (1925, p. 306) that this coalescence could not take place either in ontogeny or phylogeny until after ankylosis along the facial suture. He assumed that the post-ocular branch of the facial

suture represented a segmental boundary (a widely held hypothesis in trilobite literature) and considered (1925, p. 310) that the three pairs of head spines "all are constant morphological entities having definite mutual relations"; this led him to visualise for all trilobites a common ancestor having all three pairs of spines (but no one pair dominant) with a median occipital spine in addition (the "heptacephalic stage").¹

Should the posterior spine pair become dominant in the adult trilobite, Raw supposed that the trilobite would be primitive in its spine distribution and proparian; should the middle pair revolve postero-laterally and become dominant the form would be opisthoparian; but if the pre-ocular branches of the facial suture swung backwards (with the eyes as pivots) taking with them the anterior pair of spines to attain dominance and occupy a postero-lateral position then the trilobite would give the condition which he claimed exists in the Mesonacidae; but that this mesonacid condition should occur, the facial sutures should first become fused.

Criticisms of Raw's concept have mainly been directed against any phylogenetic significance being necessarily attached to spines since (a) these are likely to be coenogenetic structures (Swinerton in Raw, 1925, p. 322; Raymond, 1928, p. 168) and (b) their development most probably is due to purely mechanical causes for Beecher's (1898) analytical study of spines showed that external stimuli may play a large part in such development in exposed places (Raymond, 1928). The primary hypothesis that all three spine pairs "are constant morphological entities having definite mutual relations" is perhaps built upon too slender a basis of fact, particularly in view of the homologous structures brought together by both Walcott and Raw. The latter stated, for instance (1925, p. 289): "Being the pleural spines of the occipital segment—the least modified of all the head segments—the metacranial [= intergenal] spines are primitively in front of, and parallel to, the pleural spines of the adjoining first thoracic segment, and even when their directions change (as in adult *Holmia* [a mesonacid], '*Hydrocephalus carens*' [an opisthoparian] etc.), both pairs spring from points at the same distance from the axis." Raw, therefore, believed not merely that both mesonacid intergenal spines and opisthoparian fixed cheek spines are homologous, an opinion first suggested by Linnarsson (1877), but that both originated from the pleurae of the occipital, or most posterior cephalic, segment (see also Raw, 1925, p. 232). It would appear, however, from figures given of mesonacid early stages by Kiaer (1916, Pl. VI, fig. 1) of *Holmia kjerulfi* (Linnarsson), by Poulsen (1932, Pl. XI, fig. 7) of *Paedeumias hansenii* Poulsen, and by Walcott (1910, Pl. XXV, figs. 1 (*pars*), 2 (*pars*), 4, 9 and 13) of *Elliptocephala asaphoides* Emmons, of *Wanneria* Walcott (Walcott, 1910, Pl. XXXI, fig. 8), "*Olenellus*" *gilberti* Meek (Walcott, 1910, Pl. XXXVI, fig. 12, Pl. XLIII, fig. 6), *Olenelloides armatus* Peach (Walcott, 1910, Pl. XL, fig. 2), that this mesonacid intergenal spine

¹ Raw (1925) claimed that the seven-spined cephalon seen in the early stage of the Tremadoc species *Leptoplastus salteri* (Callaway) possessed spines homologous to these mesonacid so-called ancestral structures. It is perhaps remarkable that, other than in Mesonacidae and in this species of *Leptoplastus*, no trilobite ontogeny yet described shows the three pairs of marginal cephalic spines; protaspid stages are known, moreover, of some dozen non-mesonacid species occurring in the time interval between the Lower Cambrian and late Tremadoc beds.

took origin, not as Raw stated from the occipital segment but as Walcott (1910, p. 238) and Kiaer (1916, p. 66) themselves affirmed, from the pre-occipital glabellar lobe. This spine appeared, in fact (as shown in Fig. 8), as part of the larval cephalon before the occipital segment was differentiated from the telson (Warburg, 1925, p. 21).

The "intergenal" (=fixigenal, Richter, 1932) spines of certain Cambrian opisthoparian trilobites (*Paradoxides* = *Hydrocephalus*, *Zacanthoides*, etc.), homologised by Raw with the posterior, truly intergenal, mesonacid spine and the genal (=fixigenal) spines of *Proparia*, appear from published ontogenetic studies to arise not from the pre-occipital but from the occipital segment (Barrande, 1852, Pl. XLIX; Chouff, 1926, Pls. I and III). Generally the tracing of cephalic spines to glabellar segments is not convincingly effected in trilobite studies, but it is here suggested that enough evidence exists to warrant the above disagreement with Raw's statement, particularly as it is usually considered that the mesonacid cephalon has an adult occipital segment or lobe homologous with that of the paradoxidid—in some of the species of either of these families, traces of six lobes may be counted on the glabella (including the occipital lobe). Of the remaining two pairs of mesonacid cephalic spines, claimed by Raw (1925, p. 232) to pertain respectively to "the first dorsal", and combined third and fourth dorsal segments, it may well be, as Warburg (1925, p. 34) has remarked, that since "... spines other than pleural [=segmental] spines very often occur in the trilobites,¹ those mentioned need not necessarily have had that character..." (see also Fig. 4 here).

It is thought, therefore, that the case has not been proved for the relegation of the Mesonacidae to an advanced position in trilobite phylogeny. It is possible to conclude from ontogenetic studies of mesonacids that cephalisation is here more primitive than in most other families, for not only the axial lobe appears to be segmentally divided but also there is some such division on the two lateral lobes; this last has been claimed to indicate that at least some of the ancestrally individual somites combined to form the cephalon. Again the appearance here of the laterally spinose pre-occipital segment, which is followed later in the development of the individual by a new [occipital] segment set free from the anterior boundary of the telson to become incorporated into the meraspid and adult cephalon, may be taken as a further indication of primitiveness.

Trilobites are unique among Arthropoda in being typically terminated (in front and behind) by dorsal shields composed of segments dorsally fused. Trilobite caudalisation is well known to influence a varying number of segments in different genera. This caudalisation or formation of a pygidium is, as far as is known, little more than the dorsal skeletal fusion of a telsonic or anal somite with various similar but usually progressively larger pre-telsonic somites. It has been shown in certain Cambrian and later species (*Agnostus* by Barrande, 1852; *Triarthrus* by Beecher, 1896; *Shumardia* by Stubblefield, 1926) that those somites which become thoracic somites of the adult are first observed in a larval or transitory pygidium where they have been proved to have been formed one by one from the front margin of the telsonic somite, later to be released one by one from the front margin of the

¹ As for instance, in cephalae of Odontopleuridae and Cheiruridae.

transitory pygidium until when the adult thoracic numerical complement is reached the most recently formed somites are retained in a state of dorsal fusion with the telson as the pygidium and, in some cases, new somites are then added within this fused assemblage. Such caudalisation has been claimed as a progressive character among various trilobite lineages, and if it can be assumed that trilobites evolved from a non-caudalised ancestor it is likely that stratigraphically early trilobites with a pygidium composed of only one somite (the telson or anal somite) possess in that respect a primitive character. The family Mesonacidae satisfies this condition, for with the exception of the genus *Holmia* Matthew and possibly *Schmidtellus* Moberg the pygidium appears to be composed of a solitary somite. It does not appear to be recorded that a multi-somite larval pygidium exists in the family, but judging from available material this appears unlikely in most species, and the posterior or most recently formed thoracic segments are dorso-laterally furnished with little more than spinose outgrowths. The mesonacids are, in the respects enumerated above, thought to be primitive, but though it is likely, it cannot yet be regarded as proved that the absence here of a facial suture is a primitive feature, for Lower Cambrian families other than Mesonacidae are known in which opisthoparian facial sutures are already developed. In the meantime and awaiting further results it seems best to consider the family as a subgroup of the trilobite class separate from the rest as did Poulsen (1927).

(iii) *Proparia and Opisthoparia, their interrelationships*

Swinnerton (1915, p. 539) considered that the facial suture had been evolved at least three times in trilobite phylogeny, (1) in the suborder Mesonacida, which included the (facial) sutureless Mesonacidae and truly opisthoparian families believed to be descendants from the mesonacids (see *infra*, p. 435), (2) the Conocoryphidae, and perhaps other opisthoparians, (3) the Proparia; but since the time when he wrote (1915, p. 494) that "the opisthoparian and proparian conditions are therefore differentially or mutually exclusive and have arisen independently", new observations have been published.

Poulsen (1923, pp. 58, 83) made the important discovery that the developmental history of an opisthoparian trilobite, *Peltura scarabaeoides* (Wahlenberg), showed a proparian suture in its early (nepionic) stages, and later (1927, p. 330) he was able to quote further examples of similar conditions in the developmental histories of other Cambrian trilobites including the well-known ontogeny of *Sao hirsuta* Barrande, of which misleading illustrations had been published by Beecher (see Warburg, 1925, pp. 29, 32). T. Strand (1927) confirmed Poulsen's conclusions from observations on the ontogeny of *Olenus gibbosus* (Wahlenberg) and *Liostracus linmarssoni* Brögger; again more recently, Lalicker (1935) has provided a further illustration from the development of *Blainia gregaria* ? Walcott.¹ As a consequence of these discoveries the following statements made by Swinnerton (1915, pp. 494 and 493) no longer hold: (1) "In Opisthoparia the [facial] suture becomes visible

¹ Lalicker's deduction that, in the *Blainia* ontogeny, facial sutures appear before eyes, seems to be based on inadequate data and a possible misinterpretation of part of the axial furrows as facial sutures.

for the first time at the postero-lateral margin of the head shield; in Proparia, on the contrary, it appears at the antero-lateral margin." (2) "The development of the free cheek follows quite different lines in the two orders [Pro- and Opisthoparia]."

Swinnerton (1915, p. 542) had discussed the Proparia as comprising the families Encrinuridae, Cheiruridae and Phacopidae (all of which appear first in the early Ordovician except the Cheiruridae which appears in the latest Cambrian or Tremadoc beds) with the Cambrian families Burlingiidae and with reserve the Cambro-Ordovician family Agnostidae. Of the proparian genus *Burlingia* Walcott,

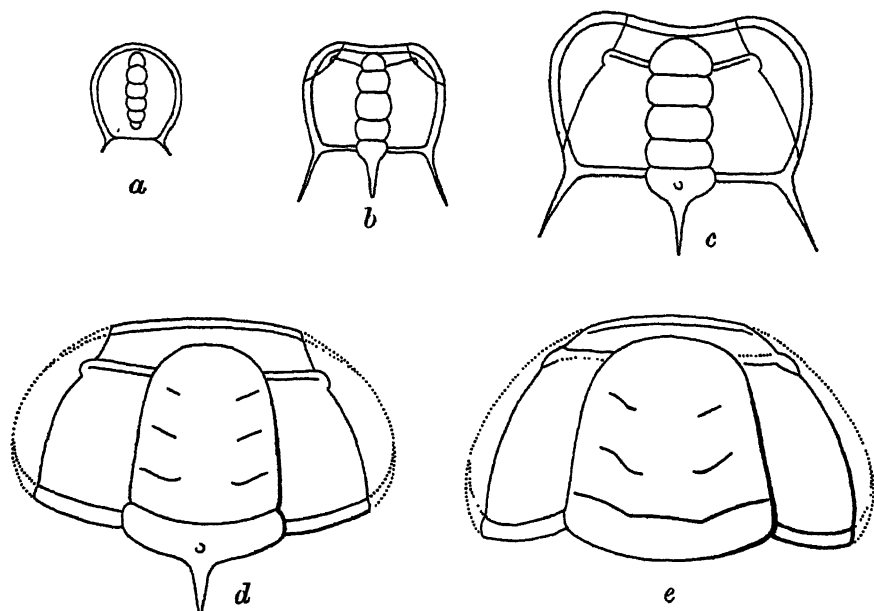


Fig. 9. Some developmental stages of the opisthoparian trilobite, *Peltura scarabaeoides* (Wahlenberg) from the Upper Cambrian of Bornholm, redrawn from Poulsen (1923). *a*. Protaspis stage; complete shield with the last axial ring belonging to the telsonic somite, $\times 20$. *b*. Cephalon of nepionic (early meraspis) stage with proparian facial suture and fixigenal spines, $\times 16$. *c*. Cephalon of later nepionic (meraspis) stage, facial suture moving backwards, $\times 16$. *d*. Cephalon of neanic (late meraspis or early holaspis) stage with opisthoparian facial suture; free cheeks restored in perspective, $\times 12$. *e*. Adult cephalon of holaspis stage; free cheeks restored in perspective, $\times 6$.

Swinnerton (1915, p. 545) wrote that intersections of the margins by the facial sutures must "be regarded as [in] the primeval positions, and can only have developed directly from the protoparous condition without the intervention of an opisthoparian stage". He excluded from the order the Calymenidae, which family was considered by Beecher to be proparian, but, since the facial sutures cut the genal angles, Beecher thought the family to be a link between the Proparia and the Opisthoparia. European writers, remembering that *Pharostoma* [*Calymene*] *pulchrum* (Barrande) has a definitely opisthoparian suture¹ and a free cheek (librigenal, Richter, 1932) spine, have usually tended to classify the family as opisthoparian; in the

¹ Beyrich (1846, Pl. II, fig. 6) and Jaekel (1901, p. 157, Text-fig. 17) incorrectly figured and Raymond (1917, p. 210) incorrectly stated that this species had proparian sutures. Barrande's work (1852) and a study of other specimens proclaim these statements to be inaccurate.

American species *Calymene callicephala* Green, *C. meeki* Foerste and early stages of *C. senaria* Auctt., however, a fixed cheek ("fixigenal", Richter, 1932) spine is developed which has led American writers to describe the family as being proparian. Swinnerton, however, placed the Calymenidae as Opisthoparia derived from other Opisthoparia (Ptychopariidae). It may be here recalled that in other families, the post-ocular branches of the facial suture tend to migrate towards the genal angles when the genal spines become obsolete (e.g. Triarthridae and Encrinuridae).

A year after the publication of Swinnerton's classification, Walcott (1916) added to the Proparia the newly discovered Cambrian families Menomoniidae (with three new genera) and the Norwoodiidae, also (1916a) the Eodiscidae because of his new genus *Pagetia*; Miss Warburg (1925, pp. 75, 78), however, rightly drew attention to the dissimilarity between these four Cambrian families collectively (i.e. the three mentioned here with the Burlingiidae) and the three stratigraphically younger families Cheiruridae, Encrinuridae and Phacopidae, and stated that the latter group could not possibly be regarded as descendants of any of the former. She remarked on the small size of the individuals comprising the Norwoodiidae and the Burlingiidae and suggested that, because of their smallness, the burlingiids might be immature forms of other trilobites. Of the Menomoniidae (a group in which the post-ocular branch of the facial suture cuts the genal angle) she wrote (1925, p. 74) that they were more probably Opisthoparia and members of the same stock as were the Ptychopariidae (an opinion supported by Richter, 1932), whereas Raw (1925, p. 315) has further proposed that not only the menomoniid genera *Menomonina* and *Dresbachia* but also the Norwoodiidae are closely related to the Ptychopariidae, though the norwoodiids show a good proparian suture. Raw also suggested that the menomoniid genus *Millardia* be related to the Conocoryphidae. It might also be noticed that Broili (1924) placed *Norwoodia* in the Olenidae and all the menomoniids in the Conocoryphidae. Neither Raw (1925, pp. 315-16), who believed that divergencies of glabella plan predated those of the cephalon plan, nor Warburg (1925, p. 78)¹ were disposed to view the Opisthoparia and Proparia as natural groups from a phylogenetic standpoint and Öpik (1929) reached a similar conclusion from entirely different reasons (more directly connected with the supposed bionomics of the animals).

If the Menomoniidae be excluded from the Proparia as Warburg and Richter recommend, then the three remaining Cambrian proparian families are the Burlingiidae, Norwoodiidae, and the Eodiscidae. Excluding also the facial sutureless genera of the Eodiscidae, since they do not materially affect the argument, it may be stated that no specimen of a "complete" individual member of these families has been recorded having a length measurement exceeding 12 mm. An isolated cranidium of the Swedish Upper Cambrian burlingiid, *Schmalenseeia*, has been recorded by Westergård (1922) with a length measurement of 6 mm.; this cranidium

¹ Warburg's statement (1925, p. 77), that similarly constructed exopodites implied that the Odontopleuridae and Calymenidae were more closely related to the Cheiruridae than to other opisthoparian trilobites, has lost its value since Raymond's conclusions that spiral exopodites are absent have been confirmed by Störmer (1933, p. 150). This does not, however, invalidate Warburg's main conclusion.

could not possibly imply a complete specimen much exceeding 12 mm. in length. Such a size is considerably below the average dimensions of an adult trilobite so that, assuming the trilobites to be of monophyletic origin, there are only two possible explanations of this dwarfed condition. The first is that these are immature forms, and with the developmental histories of *Peltura scarabaeoides* and other Cambrian opisthoparians in mind, these may just as well be immature opisthoparians as proparians. So far, however, neither in the parent rocks nor, for that matter, in any other Cambrian rocks have larger related proparians been found. The second and perhaps more probable explanation, particularly having reference to the position of the compound eyes in some of these genera, is that these forms represent examples of animals which have become sexually mature while still in a young stage, in other words, examples of neoteny or paedogenesis. Similar cases occurring elsewhere in the animal kingdom have recently been discussed by de Beer (1930, pp. 57-71). The small number of thoracic segments in the presumed adults of the two species of *Pagetia* (Eodiscidae) described by Walcott, namely two, gives additional credence to this view.

It is no innovation to suggest that arrested development or paedogenesis operated in trilobite phylogeny for Jaekel (1909, p. 391) has already put forward a similar hypothesis to explain the highly specialised condition seen in the facial sutureless members of the families Agnostidae and Eodiscidae. Here, in these families, the trilobite is apparently adult when only a very small number of thoracic segments are formed, and the animal has turned this paucity of freely articulating segments to such good account as to enable it to enroll itself in a manner perhaps unrivalled in the trilobites for safely enclosing the soft parts from extraneous attack.

Again, Warburg (1925, p. 37) has stated *à propos* of the blind Conocoryphidae, Raphiophoridae, the cheirurid *Placoparia* and the encrinurid *Dindymene*: "when during the ontogenetic development the eyes were never formed or were checked at an early stage, the growth of the free cheeks was also checked" (see also Raymond, 1935, p. 401)—so it is here claimed that conceivably in the proparian trilobites backward migration of the post-ocular branch of the facial suture was checked in development several times during trilobite phylogeny and several potentially opisthoparian lineages became proparian. Though the earliest known encrinurid, *Dindymene bohémica* Barrande, is opisthoparian, no convincing opisthoparian ancestors of the three younger proparian families have yet been postulated, but according to the Fourth Law of von Baer, namely that the young stages in the development of an animal are not like the other animals lower down in the evolutionary scale but are like the young stages of those animals (de Beer, 1930, p. 4), it is by comparing young stages of these proparians with young stages of opisthoparians that affinities may be found, provided that the essential characters are not masked by caenogenetic modifications. Raw (1925) has attempted some work on these lines.

Poulsen (1927) interpreted his observations of the proparian stage in the development of an opisthoparian trilobite as a recapitulation not of a truly proparian ancestry for the group but of the early history of the mesonacid ancestors, a postulate previously propounded by Warburg (1925, p. 39) with the proviso "if all

other trilobites came from the same ancestral stock". Warburg suggested that the Proparia might either have branched off from the ancestral stock before the "true genal" (free cheek) spines were evolved or else at a later stage when both free cheek (librigenal) and "intergenal" spines were developed. In the latter eventuality, Warburg continued, the librigenal spines were reduced and their place taken by the "intergenal" spines.

The Warburg-Poulsen interpretation again calls forth the same objection that was urged against Raw's theory of phylogeny (see pp. 425-6), namely that the evidence for homologising the fixigenal proparian spine with the intergenal mesonacid spine is unsound.

Pre-ocular branches of the facial suture in either the Proparia or Opisthoparia may be united together and contain a relic of a former rostral suture; for this reason, if for no other, it is unsafe to argue that these are homologous throughout those groups. The ocular and post-ocular branches, however, may well be homologous, though not necessarily because of any connection with primary segmentation or even cephalic spines, for though the fixigenal spines of *Zacanthoides* Walcott and *Paradoxides*, may, like the fixigenal spines of proparian trilobites, be part of the metamere of the occipital segment this does not necessarily so preclude the librigenal spine of *Phillipsia* Portlock from being similarly part of that metamere. Poulsen's discovery gives some support to this belief, but the conclusion does not follow that a proparian early stage in the history of an opisthoparian trilobite should necessarily indicate a recapitulation by that trilobite of a proparian ancestry. Not a single lineage has yet been suggested showing a proparian trilobite evolving into an opisthoparian; furthermore, whilst opisthoparian trilobites are locally plentiful in Lower Cambrian strata, no undoubted proparian genera of this age have yet been described (unless Eodiscidae are to be excepted). From Middle and Upper Cambrian rocks some few minute proparians have been recorded (see p. 429), but only in Ordovician times, when trilobites reach their acme of development, and later, do proparians become plentiful. It has been stated (see p. 429) that the Ordovician proparians show no signs of close phylogenetic connection with those found in the Cambrian deposits. Although Raw (1925, p. 285) and Richter (1932, p. 851) regarded the proparian as the more primitive condition and postulated a hypothetical line of proparian trilobites predating the evolution of the Opisthoparia in pre-Cambrian times, it is here suggested that the facts are more convincingly explained by looking upon adult proparian trilobites as permanently neotenous opisthoparians. If this tendency to neoteny became apparent in various lineages as appears to be the case with a neotenous tendency to decreasing segmentation, the heterogeneous character of the Proparia is readily appreciated; certainly since it has become enlarged, the group is more evidently heterogeneous than when Pompeckj took exception to its nature and it appears to be even more polyphyletic in origin than he imagined.

The Salter-Beecher classification of trilobites seems to be doomed to failure if its purpose be interpreted as one bringing together into such tripartite grouping animals of close genetic affinity. Pompeckj's criticism that only a single character

is relied upon to determine the primary taxonomic position of the family, will, one is convinced, be found to bear good witness.

The weakness in a phylogenetic classification of the divisions Proparia and Opisthoparia is more forcibly realised when it is recalled that only the locus of the post-ocular branch of the facial suture serves to determine the group to which any trilobite belongs; and this particular branch is merely a part of one of the several trilobite cephalic sutures, each of which has evolutionary possibilities. The evidence that this branch represents the boundary or partial boundary of a primary somite is extremely hypothetical and has received undue emphasis in past discussions.

As an easily identifiable morphological grading, the terms "hypoparian", "opisthoparian" and "proparian" are useful and will probably continue to be adopted, though care is needed in the application of the term "hypoparian", but a phylogenetic classification surely requires more than a grading based on part of a single morphological character. Richter (1932, p. 850) drew attention to some of the difficulties in interpreting the paths which various morphological characters have travelled during trilobite evolution, and again of the difficulties in deciding which characters have phylogenetic significance. He discussed the form of the trilobite glabella which is generally agreed to be an important taxonomic character, mentioning that Swinnerton (1923, pp. 244-8) believed that this structure was primitively conical in outline and evolved (as a trend along different lineages) to a cylindrical shape and eventually attained the outline of an inverted cone; whilst Raw (1925, p. 316) from ontogenetic studies maintained that the glabella primitively had the outline of an inverted cone, and that evolution was towards the conical outline, but that the divergence in glabella plan occurred very early in the history of the group, predating any changes in cephalon plan (*e.g.* sutures and spines). Richter also pointed out that though Swinnerton (1923, pp. 244-8) and Warburg (1925, pp. 40-3) credited the pre-ocular branches of the facial suture with a phyletic tendency to move inwards, his own observations on proetid genera show that in *Pteroparia* and others an outward movement was also possible; likewise that many authors have taken a small pygidium composed of few segments to indicate a primitive condition, whereas in the specialised phacopid *Trimeroccephalus*, there was a tendency, as evolution proceeded, for the pygidium to decrease in size and segmentation. The exceptions provided by Richter to these generally accepted trends fall into the same category as the supposedly retrogressive evolution shown in certain Ammonite stocks (uncoiling and simplification of suture pattern), since both the trilobite genera mentioned are stratigraphically late members of their respective families, nevertheless the fact that such conditions occur does not facilitate the application of these characters to a phylogenetic classification.

In the present state of knowledge, such a classification would probably best be obtained by linking some of the families one with another where the evidence of affinity is forthcoming, and await further research to complete the scheme. Generally speaking it will probably be found that the safest criteria of affinity are the collective characters developed in the axial region of the shield, for it is reasonable to assume that in this region lie the alimentary canal and the controlling

muscles and nerves of the organs of feeding and locomotion. Even these criteria are far from reliable, for a sudden change of habitat or habit may bring with it modifications in the available exoskeleton which mask the axial signs of affinity, so that attention must also be drawn to other characters.

(iv) *The grouping of trilobites into superfamilies and suborders*

Something of this nature was attempted by many nineteenth-century writers including E. J. Chapman (1890) and the practice was revived by Swinnerton (1915, p. 542); but later Warburg (1925) and Poulsen (1927) disputed and revised some of Swinnerton's groupings, whilst Richter has published an emended scheme of suborders incorporating new opinions—but much of this grouping is admittedly tentative (1932, p. 851).

Some thirty-eight families are dealt with by Richter; treatment is, however, omitted of the following families amongst others: Anomocaridae Poulsen (viewed by its author as an offshoot from the Ptychopariid stock); Asaphiscidae Raymond (erected to receive asaphid-like forms with 7–10 thoracic segments and a tapering glabella); Bathyuridae Walcott emend. Raymond; Dionididae Raymond; Endymionidae Raymond (erected for hypoparian forms somewhat resembling the Raphiophoridae); Entomaspidae Ulrich (for forms claimed to resemble the Harpidae, Trinucleidae and Raphiophoridae); Holotrachelidae Warburg (comprising the genus *Holotrachelus* Linnarsson, variously claimed as an asaphid, bathyurid, cheirurid, homalonotid, illaenid); Isocolidae Angelin; Leiostegidae Bradley; Plethopeltidae Raymond (a series of smooth-surfaced trilobites said to have descended from the Ellipsocephalidae); Styginidae (first erected in 1893 by Vogdes and later in 1925 considered independently by Warburg and Raymond to be allied to the Goldiidae and Illaenidae and possibly linking these to the Asaphidae); Telephidae Angelin (Ulrich, 1930*a*, pp. 6–9, could not accept Raymond's relegation of the genus *Telephus* Barrande to the Cyclopygidae but included it in the Telephidae with Raymond's odontopleurid genus *Glaphurus*); and the Tsinanidae Kobayashi. Further research may decide with which families these will ultimately be linked.

Since both Swinnerton and Richter believed in the validity of the ordinal groups Proparia and Opisthoparia, their suborders and superfamilies with the suggested interrelationships are arranged within these, though Swinnerton with some justification left the proparian families uncombined into superfamilies. He divided the Opisthoparia into four suborders: (1) Mesonacida, (2) Conocoryphida (comprising the sections, Conocoryphina, Calymmenina, Olenina and Ptychoparina), (3) Trinucleida, and (4) Odontopleurida. Richter, on the other hand, divided that order into two major divisions or suborders:¹ (1) Redlichina (comprising the superfamilies Redlichiidea, Zacanthoididea, Bathyuriscidea and Dikelocephalidea) in which division two tendencies were claimed to be marked, *i.e.* the

¹ H. Schmidt (1935) has since emended Richter's scheme, establishing three orders of trilobites—*i.e.* Telsososa (=Redlichina Richter), Telsophthina (=Ptychoparina Richter) and Proparia—the order Opisthoparia is thus abandoned by Schmidt.

pygidium tended to increase its surface area without appreciably increasing in segmentation and this was accompanied by a tendency, developed as early as Cambrian times, to a reduction in number of the thoracic segments. Richter's suborder (2) Ptychoparina (comprising the superfamilies Ellipsocephalidea, Cryptolithidea, Ptychopariidea and Calymenidea) was claimed to be characterised by a decreasing numerical thoracic segmentation and an increasing pygidial segmentation but these tendencies were less rapidly achieved than in the Redlichina and were stated to be "erst vom Devon an bemerkbarer" (1932, p. 854). With this corollary the position in this suborder of the Cryptolithidea would seem to be anomalous, a feature apparently noticed by Richter.

Richter's Ptychoparina includes therefore the families collected into Swinnerton's Conocoryphida (excepting the families Asaphidae, Dikellocephalidae, Illaenidae and Oryctocephalidae) and the Trinucleida (excepting the family Cyclopygidae; whilst the Ellipsocephalidae and Harpidae were rightly removed from this suborder and placed elsewhere in the Ptychoparina). Since Swinnerton believed the Conocoryphidae to be a primitive family, and Richter held opposite and perhaps more probable views, some divergence was here to be expected.

The Redlichina includes the constituent families of the combinations called by Swinnerton Mesonacida and Odontopleurida, with the addition of the families mentioned above as being excluded from the Ptychoparina.

The Ptychoparina, it would appear from the table accompanying Richter's classification (1932, p. 855, Fig. 43), evolved from a root stock which had previously sent off the proparian plexus and one which was subsequently expected to culminate in the Redlichina.

Gürich (1907) used as a primary ordinal character of a trilobite classification, whether or not the trilobite had many or few post-cephalic segments—then as a secondary character—the size of the pygidium in relation to the thorax as a whole. He was criticised by Swinnerton (1915, p. 494) because these characters used were progressive rather than static, and, as such, should have no place in the primary division of a group, but should merely show the path of evolution within the various primary divisions. Both Swinnerton¹ and Richter were content to regard the locus of the post-ocular branch of the facial suture as a static character and therefore of high taxonomic value (a view which is here called to question), but both have used the characters employed by Gürich in determining the evolutionary paths within their primary groups. Richter differs, however, by drawing attention to an inconsistency in the relation between segmentation and the size of the pygidium.

The validity of Richter's two opisthoparian divisions unfortunately cannot be assessed until the lineages and groupings suggested within those divisions are placed on a more reliable basis. As an instance it may be noted that many writers since the time of Barrande (1852) and Holm (1886) have considered that the Goldiidae and Illaenidae are closely related, for the respective species not infrequently have closely similar hypostomes, rostra and pygidia, but it is as yet at least doubtful whether these families arose from a redlichiid or ptychopariid stock. Ulrich (1929,

¹ Warburg (1925, p. 54) pointed out that the course of the facial suture was a progressive character within Swinnerton's order Protoparia.

pp. 59-63), whose long experience in palaeozoic palaeontology makes his views valuable, warns us that in his opinion prevailing conceptions of the systematic relations of the Ordovician and older trilobites are "largely based on overworked theories, weakly grounded deductions and pure assumptions employed indiscriminately as though they were established facts and immutable laws". He advises that the facts themselves be worked out, and that it be remembered that "present effort to classify them [the trilobites] into families and groups of higher rank can be nothing better than a provisional arrangement". It is thus perhaps not surprising that the German revisers of Zittel's Grundzüge, have continued to adopt only family divisions among the trilobites.

V. APPENDIX

(i) *Richter's grouping of the Proparia*¹

Superfamilies	Family ²	Range ³
I. Eodiscidea	Agnostidae McCoy Eodiscidae Raymond [olim Microdiscidae]	M. Camb.-U. Ord. L. Camb.-M. Camb.
II. Norwoodiidea	Norwoodiidae Walcott	U. Camb.
III. Burlingiidea	Burlingiidae Walcott	M. Camb.-U. Camb.
IV. Phacopidea ⁴	Cheiruridae Corda Encrinuridae Angelin Phacopidae Corda	Trem.-M. Dev. L. Ord.-U. Sil. L. Ord.-L. Carb.

(ii) *Comparative grouping of the Opisthoparia by Richter and Swinnerton*

Richter's superfamilies	Family	Time range	Swinnerton's groups
I. Redlichiidea	Mesonacidae Walcott Paradoxididae Emmrich Redlichiidae Poulsen	L. Camb. M. Camb. L. Camb.-M. Camb.	Mesonacida " "
II. Zacanthoididea	Ceratopygidae Linnarsson Lichidae Corda Odontopleuridae Burmeister [olim Acidaspidae Barrande] Oryctocephalidae Beecher Remopleuridae Corda Zacanthoididae Swinnerton	Tremadoc ⁵ Trem.-U. Dev. L. Ord.-U. Dev. L. Camb.-M. Camb. U. Camb.-U. Ord. L. Camb.-M. Camb.?	Not placed Odontopleurida " Olenina Mesonacida "
III. Bathyriscidea	Bathyriscidae Rud. Richter ⁶ Corynexochidae Angelin Illaenidae Corda Scutellidae Rud. & E. Richter ⁷ [Bronteidae Corda]	M. Camb. L. Camb.-M. Camb. L. Ord.-U. Sil. M. Ord.-U. Dev.	Ptychoparina Not placed Ptychoparina Odontopleurida
IV. Dikel[?]ocephalidea	Asaphidae Burmeister Cyclopygidae Raymond [olim Aeglinidae Pictet] Dikellocephalidae Linnarsson Symphysuridae Poulsen ⁸ (really Nileidae Angelin)	U. Camb.-U. Ord. ⁸ Trem.-U. Ord. U. Camb.-Trem. Trem.-U. Ord.?	Ptychoparina Trinucleida Ptychoparina "
V. Ellipsocephalidea	Ellipsocephalidae Matthew Olenidae Burmeister Otariionidae R. & E. Richter [olim Cyphaspidae Salter] Proetidae Corda	L. Camb.-M. Camb. U. Camb.-Trem. ¹⁰ U. Ord.-Carb. U. Ord.-Perm.	Trinucleida Olenina Olenina (sub. Proetidae) Olenina

Richter's superfamilies	Family	Time range	Swannerton's groups
VI. Cryptolithidea	Cryptolithidae Angelin	Trem.-U. Ord.	Trinucleida
	[olim Trinucleidae Emmrich]		
	Raphiophoridae Angelin	L. Ord.-U. Sil.	"
	[= Ampyxidae E. J. Chapman]		
VII. Ptychopariidea	Shumardiidae Lake ¹¹	U. Camb.-U. Ord.	" ?
	Conocoryphidae Angelin	L. Camb.-Trem.?	Conocoryphina
	Harpidae Barrande ¹²	L. Ord.-U. Dev.	Trinucleida
	Ptychopariidae Matthew ¹³	L. Camb.-Trem.?	Ptychoparina
	Solenopleuridae Angelin	L. Camb.-M. Camb.	"
VIII. Calymenidea	Calymenidae H. M. Edwards	L. Ord.-M. Dev.	Calymmenina
	Homalonotidae E. J. Chapman	L. Ord.-U. Dev.	"
	Menomoniidae Walcott	U. Camb.	Not placed

(iii) Notes on the above groupings (i) and (ii)

¹ Swinnerton did not combine together any families as subgroups of the Proparia.

² The families are here arranged alphabetically within the suborders.

³ The ranges given here are not necessarily the same as those stated by Richter (1932, p. 855).

⁴ This suborder is Salter's unaltered group Phacopini (see p. 415).

The members of the Phacopidae do not appear to have any special morphological character other than the post-ocular course of the facial sutures which could cause them to be linked with the other families of this suborder.

⁵ Richter followed Raymond (1913) who included the Middle Cambrian genus *Albertella* Walcott in the Ceratopygidae, and thus gave the family an extended time as well as geographical range. Swinnerton, however, placed this genus in the Zacanthoididae. Warburg (1925, p. 56) considered that both *Albertella* and *Zacanthoides* might have descended from a *Redlichia*-like ancestor, and *Ceratopyge* in turn from an *Albertella*-like ancestor. The time interval between the presence of these two latter genera is, however, considerable and the resemblances too superficial to be important.

⁶ Swinnerton followed Raymond (1913) by including the genus *Bathyriscus* Meek in the Bathyriscidae Walcott along with Upper Cambrian and Ordovician genera. Walcott (1916a, p. 309), however, transferred this to the Corynexochidae, in which family it was retained by Broili (1924) and Warburg (1925).

⁷ R. & E. Richter (1925, p. 239) revived on grounds of priority the name *Scutellum* Pusch, 1833 to replace *Goldius* de Koninck, 1841 and *Bronteus* Goldfuss, 1843. They then erected the family Scutellidae. Poulsen (1934, p. 27) announced that the family name Scutellidae Agassiz held precedence for a group of Echinoidea containing the genus *Scutella* Lamarck, 1816, and used the name Goldiidae Raymond for this trilobite family. It would seem expedient, therefore, not to use the name *Scutellum* for trilobites.

⁸ Swinnerton included the Middle Cambrian genus *Ogygopsis* Walcott in the Asaphidae as did Walcott and Raymond. Warburg and Richter, however, have since excluded it on adequate grounds.

⁹ Angelin (1854) used the name Nileidae for a family including the genera *Nileus* Dalman, 1827, and *Symphysurus* Goldfuss, 1843, a precedent followed by Brögger (1886), though Brögger considered the Nileidae as a subfamily of the Asaphidae—this name, therefore, would appear to take precedence over the name Symphysuridae Poulsen, 1927, for a similarly constituted family.

¹⁰ Richter quoted the range of the Olenidae as L. Camb.-Mid. Devonian. He included in this family the genus *Aulacopleura* Corda [range Ord.-Mid. Dev.], which placing is disputed by various authors including Warburg and Poulsen who would follow Oehlert and place it in the Cyphaspidae (= Otariionidae); it is here thought best to revive the family Aulacopleuridae Angelin to accommodate this genus. Richter followed Swinnerton in considering the genus *Protoleus* Matthew as an olenid; that olenids came from a *Protoleus*-like stock is possible but for the present that genus should perhaps be included in the Ellipsocephalidae.

¹¹ Though it is believed that Richter's suborder Cryptolithidea is a more homogeneous unit than Swinnerton's Trinucleida there seems but slender justification for including here the Shumardiidae. Such supposed resemblances as occur can but be due to homoeomorphy. Broili (1924) following Pompeckj placed the genus *Shumardia* Billings with reserve in the Conocoryphidae, but this last family without this addition probably represents a polyphyletic plexus (see p. 419).

¹² The family name Harpididae Corda is synonymous with and has priority over Harpidae Barrande, but since there is doubt (Chapman, 1890; Poulsen, 1927; and others) as to whether *Harpides* Beyrich and *Harpes* Goldfuss really belong to the same family it is thought best to retain both family names in an emended condition. Oehlert (1886, p. 123) has drawn attention to the

resemblances between *Aulacopleura* Corda and *Harpes* Goldfuss; superficially this line of thought appears attractive.

¹³ Swinnerton included the genus *Triarthrus* Green (range Trem.-U. Ord.) in this family, Warburg (1925, p. 61), however, would retain it in the Olenidae, but Ulrich (1930, p. 214) would associate it with other "olenid" forms such as *Acerocare*, *Cyclognathus*, *Parabolinella* and *Peltura* in the family Triarthridae E. J. Chapman emend. Ulrich. The resemblance between *Triarthrus* (both in the adult and protaspis) and *Peltura* H. M. Edwards rather than with *Olenus* (*sensu stricto*) adds confirmation to Ulrich's view. The genera *Bavarilla* Barrande and *Euloma* Angelin are also considered to be doubtfully placed in the Ptychopariidae which family seems to show close affinities with the Solenopleuridae.

VI. SUMMARY

1. The cephalic sutures are defined; their distribution and interrelationships discussed. Few facts are available concerning the evolutionary movements of these, but various theoretical deductions have been made.

2. Opinion on the supposed function of these sutures is summarised and it is concluded that the sutures probably did not exist solely for the purpose of ecdysis.

3. Consideration is given to the historical aspect of trilobite classification into three orders where the ordinal character is the course taken by some of the cephalic sutures. This classification has been adversely criticised; attention is called to the fallacy of assuming that the chosen cephalic sutures were:

- (a) relics of primary segmentation,
- (b) homologous in all groups,
- (c) in themselves alone of phylogenetic significance.

4. Three orders have been erected in turn to accommodate, according to their founders, the most primitive trilobites; none contains a single family in common with another of the orders. Pompeckj's and Swinnerton's objections to Beecher's order Hypoparia are upheld, as are Warburg's and Rud. Richter's objections to Swinnerton's order Protoparia.

5. The order Mesonacida (as defined by Poulsen) has a unique position; Poulsen regarded it as the most primitive group of known trilobites; Raw, as the most specialised group at least as far as the cephalic "segmental" spines and sutural evolution is concerned. Disagreement is expressed with this opinion of Raw's, essentially because of a more probable interpretation of the particular segmental origin of one series of spines homologised by Raw, and because there are doubts whether the remaining two spine pairs are necessarily of metameric (segmental) origin.

6. Though absolute proof is as yet lacking, Swinnerton and Poulsen are thought to have justifiably stated that the absence of true facial sutures in Mesonacidae is primary; some of the supposed primitive features of the family (or order using Poulsen's restricted sense) are discussed.

7. The bearing of recent ontogenetic work on the relationship of Proparia and Opisthoparia suggests that the proparian condition may be regarded as arrested development, and therefore previous failure to recognise this has resulted in the establishment of a group here held to be polyphyletic.

8. A satisfactory classification of the group might be evolved, as sufficient reliable knowledge accumulates, by combining allied families into superfamilies. Two attempts at this form of classification are discussed and summarised; these however are based on the primary ordinal value of the supposed (adult) static condition of part of one of the cephalic sutures, and can only be regarded as provisional.

I would express my indebtedness to Dr O. M. B. Bulman and to Dr J. Pringle with whom I have discussed various subjects in this review; to the pen of the former, I owe the drawings reproduced here as Figs. 1 and 4-9.

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ADDENDUM

In January 1936, while this article was passing through the press, copies arrived in London of a paper by T. Kobayashi published in Japan on 20th November, 1935, entitled "The Cambro-Ordovician Formations and Faunas of South Chosen. Palaeontology. Part III. Cambrian Faunas..." (*J. Fac. Sci. Tokyo Univ.* **11**, **4**, 49-344). The author reviews trilobite classifications and reaches independently several of the conclusions expressed in the preceding article. He challenges the primary taxonomic importance of the facial suture and comments on the unreliability of ontogenetical evidence in classification. He also deduces (p. 95) that "the Proparians might be the terminals of evolutionary lines" and are therefore of polyphyletic origin. The paper is mainly concerned with such Cambrian trilobites as occur in the Pacific rather than only in the Atlantic faunal province, and of these the author changes many family references, erecting also nine new families, namely Crepicephalidae, Damesellidae, Emmrichellidae, Komaspidae, Lancastriidae, Marjumiidae, Olenopsidae, Pagetidae and Pagodidae. These and other families described or discussed are distributed among five suborders: Agnostida (=Rud. Richter's Eodiscidae), Mesonacida (=Richter's Redlichiidae with Zacantheididae *pars* and the Burlingiidae), Corynexochida, Ptychoparida (=Richter's Ptychoparina with the Norwoodiidae) and the Dikelocephalida. The characters of these suborders do not appear to be defined, and no mention is made of the order to which they belong, but it seems (p. 84) that he would retain the order Proparia to include the Cheiruridae, Encrinuridae and Phacopidae.

In a subsequent work "On the Parabolina Fauna from province Jujuy, Argentina..." (*Jap. J. Geol. Geogr.* **13**, 1936, 89-90), Kobayashi described a new "late Upper Cambrian" genus *Jujuyaspis* which, judging from his illustrations, becomes adult and attains a length of at least 18 mm. whilst retaining proparian facial sutures. This genus, I believe, is correctly referred to the Olenidae. The aspect of the individuals and the stratigraphical age preclude from any suggestion that the genus represents an ancestor either of the typically opisthoparian Olenidae, or of any known proparian genus. It is here accepted as offering additional proof of the weakness of the groups Pro- and Opisthoparia in phylogenetic classification.

THE PHYSIOLOGICAL EFFECTS OF PRESSURE

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I. INTRODUCTION

PRESSURE changes in the environment may transmit their effects to living cells in a number of ways. The present review deals with the effects of pressure *per se*, the uncomplicated effects of which can be demonstrated only when the tissue or organism is completely immersed in a liquid through which the pressure is transmitted. It is then found experimentally that relatively high pressures are necessary in order to produce significant changes in physiological functions. It is quite otherwise in instances where the pressure is applied through the atmosphere or other gases, for the reason that the pressure changes are accompanied by a corresponding change in the tension of the gases dissolved in the protoplasm, and thus relatively slight changes in pressure may produce important effects. Complete immersion in a fluid is essential, otherwise pressure changes inevitably result in distortion and injury. When pressure is applied locally there is first a displacement of the fluid portions of the cell, followed by an irreversible disorganization of the more fixed structures. These effects are most severe at the periphery of the area of compression where the gradient is greatest. The local application of a pressure of only 200 mm. of mercury is sufficient to stop conduction in a nerve trunk within a few minutes.

The influence of increased hydrostatic pressures in altering physiological processes has a special interest on account of the fact that its action is determined by changes which are distributed uniformly throughout the substance of the cell. This is presumably a fundamental action on molecular structure and relationships, brought about by the resulting volume change. Comparatively little is known of the influence of high pressures on living systems, and at the present time one can only record a group of more or less isolated observations, with special emphasis on muscular contraction, in which field the effects of pressure have been investigated to a somewhat greater extent. The attempt has been made to include an account of all significant studies relating to the field covered by this review and, as far as possible, to give details of the results and the experimental conditions under which they were obtained.

II. PHYSICO-CHEMICAL ASPECTS

Changes in function resulting from pressure must find their explanation in relation to an influence on the physical and chemical properties of liquids. It will therefore be profitable to indicate briefly some of the more important ways in which fluid systems are affected by increased pressures. For further details the reader is referred to the excellent treatise by Bridgman (1931).

(a) *Volume.* The compressibility of liquids is commonly assumed to be unimportant, but from our present point of view the volume changes are of great significance. For example, under a pressure of 12,000 kg./cm.² (1 kg./cm.² = 0.968 atm.)¹ at room temperature pure water undergoes roughly a 20 per cent. reduction

¹ One atmosphere is thus approximately 1 kg./cm.² or 14.7 lb. in.² and 1 lb./in.² may be taken as 0.07 kg./cm.² or 0.068 atmosphere.

in volume. Liquids become less compressible as the pressure is increased. Bridgman has found that on the average the compressibility at 12,000 kg./cm.² is only one-fifteenth of that at atmospheric pressure. The effect of pressure on the volume of a liquid is influenced by a number of factors; for example, it becomes less as the temperature is raised. The addition of a soluble electrolyte to water also decreases its compressibility (Cohen & Schut, 1919).

(b) *Chemical reactions.* In general the velocity of chemical reactions in the liquid phase is increased by an increase in pressure. Observations have been made by a number of workers, but here we need mention only a recent paper by Fawcett & Gibson (1934) giving the results of studies on the influence of pressures up to 3000 atmospheres on the velocity of about 50 organic reactions. With few exceptions the result was to increase the rate of reaction from 5 to 10 times, an effect much greater than the volume changes. The various factors entering into this result are discussed by Fawcett and Gibson. The most important would appear to be the decreased space between the molecules, which would have the effect of increasing the collision rate between the reactants—a factor which would increase out of proportion to the volume changes. In reversible reactions involving a change in volume a further effect of the application of pressure is to shift the equilibrium to the side of the lesser volume.

(c) *Viscosity.* Of all the properties of liquids affected by pressure viscosity changes are of the greatest magnitude and show the widest range of variation from substance to substance. The detailed behaviour of a number of liquids at various temperatures under pressures up to 12,000 kg./cm.² has been studied by Bridgman (1926, 1931). With the exception of water and mercury, the viscosity of methyl alcohol is least affected by pressure, the increase being about 10 times for 12,000 kg./cm.², while in the case of eugenol a viscosity increase of the order of 10⁷ times is obtained. In general the pressure effect bears a direct relationship to the complexity of molecular structure. Unlike most of the effects of pressure, the viscosity increase becomes progressively greater with equal increments of pressure, so that when viscosity is plotted against pressure the curve shows a rapidly increasing upward curvature.

Water occupies an exceptional position in that it shows a decreased viscosity when compressed. This, however, is true at only relatively low pressures and temperatures. Above about 25° C. the viscosity increases as it does for other liquids at all temperatures. The addition of an electrolyte to water has the effect of causing the viscosity increase to come on at a lower temperature: Cohen, for example (1892), reports that in the case of a 3 per cent. NaCl solution a pressure of only a few thousand pounds per square inch begins to cause an increase in viscosity at 14° C.

(d) *Miscellaneous.* Pressure alters the properties of solutions in various other directions, such as solubility, ionic dissociation, and surface tension. These are effects which are almost certainly reflected in changes in the physiological properties of a tissue, but even in simple inorganic solutions the changes produced are unfortunately complex and their application to the living cell is at the present time quite impossible. In general the dissociation of an electrolyte in weak solution is

increased by pressure. Solubility becomes less or greater depending upon the system studied, and the effect of pressure on the surface tension of water is, at least in theory, to increase it.

III. GENERAL AND HISTORICAL

The physiological action of increased pressure is a problem which has interested investigators for many years. Studies and speculation have centered in the symptom complex known as caisson sickness from which divers and caisson workers were prone to suffer before the true cause of the difficulty—the formation of bubbles of nitrogen in the tissues during compression—was understood. A summary of the early investigations in this connection will be found in the monograph by Hill (1912). The matter was finally settled by the fundamental work of Paul Bert. In an extensive series of experimental studies Bert also laid the foundations for our knowledge of the effects of various gas mixtures and tensions upon animal life. These studies are described in detail in a volume published in 1878, which includes an account of the earlier work. Previous to this time and up to recent years the possibility of an effect of pressure *per se* has been discussed, with very little adequate evidence. Practically all the older work has been complicated by the presence of a gaseous phase through which the pressure was transmitted. Accompanying the pressure changes there was therefore produced a change in the concentration of the dissolved gases. Long before Bert it was known that increased pressures exerted no unfavourable influence on small animals. For example, it was shown by Muschenbroeck (1755) that the survival period for small animals in a closed system was prolonged when the pressure was increased to 3 atmospheres. Similarly Achard (1801) found that birds survived about five times as long when exposed to a pressure of 4 atmospheres as they did in the same container at atmospheric pressure. Poiseuille (1835) made direct observations on the capillary circulation in the transparent parts of frogs, salamanders, tadpoles, young rats and mice and was unable to note any effect of increased air pressures up to 8 atmospheres. In these experiments, as well as in those of Bert, in which pressures up to 20 atmospheres were employed, any changes might be due to alterations in the concentration of dissolved gases in the tissues, and no effect clearly attributable to the pressure *per se* could be observed. From the point of view of the material covered by this review by far the most important experiments are those of Regnard, the results of which were reported in a series of communications to the Société de biologie and Académie des sciences between 1884 and 1888 and also in a book, *La vie dans les eaux*, published in 1891. Impressed by the findings of the *Talisman* dredging expedition, which recovered living forms at a depth of 12,000 metres, corresponding to a pressure of about 1000 atmospheres, Regnard undertook a series of laboratory experiments with modern apparatus in the course of which the effects of hydrostatic pressure up to 1000 atmospheres were studied on a large series of living organisms ranging from yeast and Protozoa to fish and frogs. Details of some of these studies will be considered later in connection with some of the specific topics discussed.

The fact that deep-sea forms have as their normal habitat pressures great enough to produce important physiological changes and even death in organisms living near the surface is a significant observation which seems to have attracted very little attention since Regnard's pioneer studies.

IV. PROTEINS

An observation of great interest is that of Bridgman (1914), who showed that when certain proteins are exposed to high pressures they undergo a change similar to that produced by heat. On egg albumen at 20° C. a pressure of 5000 atmospheres (75,000 lb./in.²) for 30 min. gave a perceptible thickening, while 6000 atmospheres produced an appearance resembling curdled milk. At 7000 atmospheres coagulation was complete and the material was capable of standing under its own weight. Further increments of pressure up to 12,000 atmospheres brought about no further change. A pressure of 3000 atmospheres for 16 hours was practically without effect, and the changes produced by 5000 atmospheres were but slightly increased by prolonging the compression time to 1 hour. When the temperature was reduced to 0° C. the pressure effects were slightly greater. Bridgman (1925) has also noted that meat proteins are coagulated by high pressures.

The coagulating action of high pressures on proteins was independently observed at about the time of Bridgman's publication by Hite, Giddings & Weakley in some unpublished work kindly communicated to me by Prof. Giddings. It was found that whole egg-white, exposed to 100,000 lb./in.² for 4 hours, was completely coagulated, taking the shape of the glass container and adhering to it so tightly that it was impossible to get it out whole. These investigators also noted that high pressures (of the order of 100,000 lb./in.²) caused a clouding and precipitation of solutions and of the proteins in horse serum. Recently Basset, Macheboeuf & Sandor (1933) have come upon the same effect in connection with studies on the effects of high pressures on enzymes and immune sera, and their observations agree closely with those of the American workers. In the case of serum globulin and egg-white they observed the first effects at 5000 atmospheres, a pressure causing the solutions to become slightly opalescent, while at 13,000 atmospheres coagulation was complete. The maximum effects were obtained by an exposure of not more than 30 min. In contrast with these results, serum albumen was not coagulated by the highest pressures employed (17,600 atmospheres).

The fact that certain proteins are coagulated as the result of high pressure is of significance from a biological point of view, for it points clearly to certain limiting values beyond which life cannot exist. Actually, as will be shown later, the magnitude of the pressure required to precipitate proteins is of the same order as that required to kill bacteria or inactivate enzymes. As to the mechanism through which polymerization is brought about physical chemists have very little to offer. However, it is of interest to note that there is a similarity between the action of pressure and temperature, both in the change produced and in the relative unimportance of a time factor.

Another effect of pressure which unquestionably is of importance in biological systems is its influence on the hydration of colloidal systems. There is a decrease in the volume of the colloid gel plus dispersion medium as imbibition proceeds. According to the theorem of Le Chatelier, pressure, which causes a decrease in volume, should promote the imbibition of water, and Posnjak (1912), for example, has shown that gelatin gels in a water medium take up more water when compressed. The factor of increased hydration would thus play a part in the viscosity increase brought about by pressure in such systems.

V. ENZYMES AND TOXINS

(a) *Plant enzymes.* That enzymes of yeast withstand considerable elevations of pressure was first demonstrated by H. and E. Buchner (1897), who showed that the juice expressed from cells by pressures up to 500 atmospheres retained the original ability of the living yeast cells to ferment carbohydrates. In experiments begun in 1913 at the West Virginia Agricultural Experiment Station (unpublished), Hite, Giddings & Weakley showed that high pressures are capable of inactivating zymase obtained from the common brewer's yeast, *Saccharomyces cerevisiae*. For a transitory application of pressure about 45,000 lb./in.² was required; for 30 min. about 30,000 lb.; and for 60 min. about 25,000 lb. These pressures, causing inactivation of zymase, are approximately the same as those (reported by the same workers, 1914) required to kill intact yeast cells.

Working with yet higher pressures, Basset & Macheboeuf (1932), Macheboeuf, Basset & Levy (1933), and Macheboeuf & Basset (1934) in France have explored the problem further. On saccharase of yeast, pressures up to about 6000 atmospheres for 30 min. were without effect, while at 10,000 atmospheres the activity, as measured by the rate of sugar fermentation, was reduced a varying amount; in some instances all activity was lost, but in others as much as 40 per cent. was retained. Some of the factors responsible for this variability were studied. The duration of the pressure played a small part—in the example given 9000 atmospheres caused a loss of 59 per cent. of the original activity in 30 min., and 66 per cent. in 60 min. Further, a considerably greater loss in activity resulted from a given pressure at low pH values, while the effect of increasing the pH beyond the neutral point was but slight. These authors also experimented with laccases from various plant sources. The effects were essentially similar to those described for saccharase except that rather higher pressures were required to bring about a given reduction in activity.

(b) *Digestive enzymes.* The same investigators who have studied the effect of pressure on the enzymes responsible for the fermentation of sugars have made investigations on various digestive enzymes. Earlier, Regnard (1884*a*, 1891) had exposed saliva to 1000 atmospheres and noted that the conversion of starch to sugar continued, and also that this pressure had no effect on the digestive activity of gastric and pancreatic juices. Hite, Giddings & Weakley (unpublished) got complete inactivation of pepsin solutions (Parke, Davis & Co., 1:3000) after about 5 min. at

120,000 lb./in.², whereas an exposure of about 2 hours was required to produce this effect at 100,000 lb. A few observations were also made on "Pangestin" (pancreatin, Abbott Laboratories) in relation to the influence of pressure on its power to digest solutions of starch. 100,000 lb./in.² applied for 10 min. was without effect, but some reduction in activity was noted after 30 min., whereas inactivation was complete in 14 hours. These values are from tests made very soon after the release of pressure, and some increase in activity was noted after standing overnight. In connection with their experiments on the preservation of milk by pressure Hite, Giddings & Weakley (1914) have also noted that after all bacteria are destroyed certain slow changes continued to take place, changes which were believed to be due to enzymes unaffected by the high pressure. Studies were made on the activity of galactase in relation to pressure, and it was found that this enzyme was not destroyed by a pressure of 100,000 lb./in.² at room temperature for 7 days nor by the same pressure applied for an hour each day for a week. Basset, Lisbonne & Macheboeuf (1933) and Macheboeuf & Basset (1934) have reported the results of experiments made on the effects of pressure on the enzymes of pancreatic juice. For example, in one experiment in which the activity of trypsin was tested in a neutral solution it was found that compression for 45 min. at 13,500 atmospheres caused the loss of 55 per cent. of the original activity; at 16,000 atmospheres, similarly applied, a loss of 75 per cent. Still higher pressures were required to destroy the activity of the proferment, trypsinogen. Most resistant of all was pancreatic amylase, which was unaffected by 13,500 atmospheres for 30 min. but lost 66 per cent. of its activity under 15,500 atmospheres for the same period. Pancreatic lipase in the example cited was far more sensitive to pressure, being completely destroyed by 11,000 atmospheres. Thus, in general, the enzymes of the pancreatic juice withstand greater pressures than the various plant enzymes studied in the same laboratory.

With a view to obtaining tissue enzymes free from bacterial contamination, Hite & Morse (1920) have investigated the action of high pressures on the activity of the urea-splitting enzymes in liver tissue from rabbits. According to the few figures cited a complete suppression of activity occurred on exposure to 160,000 lb./in.² for 5 min., or 100,000 lb. for 1 min. However, in one case activity was still present after 16 hours' exposure to 100,000 lb., and it was unimpaired at 75,000 lb. for 5 min. While the latter pressure is distinctly greater than that required to kill non-spore-forming bacteria, the leeway was regarded as too small to give the procedure practical value in the preparation of sterile active enzyme solutions.

A point of interest in connection with these experiments is the great difference in susceptibility shown by different types of enzymes. The fact that galactase and ptyalin are not inactivated by a relatively enormous pressure suggests the existence of fundamental differences in chemical structure. It was noted by both Macheboeuf *et al.* and Hite, Giddings & Weakley that at the pressures required to kill enzymes there was always some clouding of the solution due to the precipitation of proteins, and it was possible that the enzymes might at the same time be thrown out of solution and thus account for the inactivation produced by pressure. In support

of this notion is the observation made by the latter investigators (unpublished) that ptyalin and galactase were the only enzymes studied which were not affected by 100,000 lb./in.² and they were the only enzyme solutions not precipitated by pressure. One suggestive experiment was carried out with ptyalin. In this instance it was found that the enzyme, which ordinarily is quite unaffected by 100,000 lb., was inactivated by this pressure when in a solution of egg albumen, and that some time after the removal of pressure there was a partial return of activity. It was thought probable, however, that there might also be a precipitation of enzymes independently of the associated material. Further work is required to settle the question.

(c) *Toxins and viruses.* There are a few observations on the effect of high pressures on various toxins and viruses, notably those of Basset & Macheboeuf in France. These authors (1932, 1933) were able to inactivate completely the toxins of diphtheria and tetanus by an exposure of 45 min. to a pressure of 13,500 atmospheres, a treatment which was without effect on the corresponding antitoxins. In sera, however, the antitoxic properties were completely destroyed by this treatment, which also resulted in the precipitation of a major part of the proteins. On cobra venom a pressure of 13,500 lb. for 45 min. resulted in a loss of a major part of its haemolytic property. This treatment had no effect on the properties of tuberculin. The pressure required to destroy the effectiveness of toxins is thus relatively high, far higher than that required to kill the bacteria concerned, and this fact points to a procedure for preparing immunizing antigens. Earlier Larson, Hartzell & Diehl (1918) had made such an attempt and reported that filtrates of typhoid bacteria which had been subjected to a direct load of 6000 atmospheres for 14 hours are superior to the whole culture for immunising purposes.

The action of pressure on viruses has been studied by Giddings, Allard & Hite (1929) in relation to the inactivation of tobacco-mosaic virus. The observations were made on the juice expressed from mosaic-diseased tobacco plants which was subjected to pressure for varying periods of time and later inoculated into healthy plants. Twenty control plants all remained healthy, whereas the 30 plants inoculated with the untreated juice showed 26 mosaic plants. In contrast the 40 plants inoculated with mosaic juice which had been subjected to compression at 75,000 lb./in.² for from 5 to 7 days showed 31 diseased plants, while mosaic symptoms did not develop in any plants inoculated with mosaic juice which had been treated with 130,000 lb. or more for from 1 to 2 days. These results are of interest in connection with the recent discovery of Stanley (1935), who has isolated a crystalline protein capable of causing the tobacco-mosaic disease.

Basset, Wollman, Macheboeuf & Bardach (1933) exposed a virulent strain of vaccine virus, effective for rabbits in a dilution of 1:5,000,000, to pressure and found it relatively susceptible to this treatment. 1800 atmospheres for 45 min. reduced its virulence so that to be effective it had to be inoculated in concentration of 1:1,000,000, and it was made completely inert by a pressure of 4500 atmospheres for 45 min. It has been shown by the same authors that the effectiveness of various bacteriophages is readily destroyed by pressure. In the case of the bacteriophage

of *B. staphylococcus* 1000 atmospheres for 45 min. was found to reduce its activity and 2000–3000 atmospheres to stop it completely. The bacteriophages of *B. typhosis* and *B. subtilis* were less susceptible, retaining the greater part of their activity up to 4500 atmospheres but being completely destroyed by 7000 atmospheres. In the presence of the bacteria higher pressures were required to produce a given effect.

While very little can be said regarding the mechanics involved, it is well established by the experiments described above that the activity of most, if not all, biological processes is destroyed when pressure is sufficiently increased. In view of the fact that high pressures increase the rate of many chemical reactions and because lesser pressures have been shown to have a stimulating action on certain physiological processes, the study of the influence of moderate pressures on the activity of enzymes, etc., becomes a matter of great interest. The only observations of this character are those of Lumière & Couturier (1927), who studied the effects of hydrostatic pressures up to 100 kg./cm.² on the activity of natural haemolysins. During the first hour there was a well-marked increase in activity, followed later by a gradual decrease and a return to the activity of the controls. Inactivated rabbit anti-sheep serum gave results similar to those obtained with normal serum.

VI. UNICELLULAR ORGANISMS

Among the earliest experiments on the effects of increased hydrostatic pressures on unicellular organisms are those of Certes, who in 1884 reported the failure of a pressure of 600 atmospheres for 24 hours to influence the pathogenicity of the bacillus of anthrax. With Cochin (1884) he also reported that yeast cells exposed to a pressure of from 300 to 400 atmospheres for several days continued to ferment sugar during this period. In contrast to these findings Regnard (1891, p. 150) reports that a pressure of 600 atmospheres stopped all fermentation but that it proceeded normally soon after decompression. Even at 1000 atmospheres complete recovery occurred. Regnard also studied the influence of the prolonged application of pressure on the putrefaction occurring in a number of substances. Usually a pressure of 700 atmospheres was employed which was allowed to act from 2 to 5 weeks. The following substances were included in the study: egg-white and yolk, milk, urine, sugared extract of yeast, meat, and blood, all contaminated with putrefactive organisms. In every instance after the period of compression the substance was found to be well preserved, while the controls were in a state of advanced decomposition. In some cases fewer organisms were present following pressure treatment, indicating a lethal action which does not accord with the experience of other workers at such comparatively low pressures. Certes (1884*a*) has suggested that the products of metabolism in the closed container may have been in part responsible. On the other hand, these experiments are the only ones known to the writer in which the prolonged action of sublethal pressures on bacteria has been studied, and it is of great interest that 700 atmospheres, applied over a long period of time, inhibited bacterial activity. E. Buchner (1897) employed the hydraulic

press in his experiments demonstrating the independent activity of zymase and they were extended by H. Buchner (1897), using pressure between 400 and 500 atmospheres on both yeast and bacteria. The expressed fluid from the yeast had the ability to ferment sugar, while that from bacteria retained the toxic properties of the intact organisms. Hill (1912, p. 116) reported that *B. coli* withstood 1700 atmospheres for 10 min.

As we now know, the pressures employed by these early workers were too low to cause destruction of bacteria, and it has been only within recent years that this goal has been attained. Pressures having a definite influence on bacteria were first employed by Chlopin & Tammann (1903), who reached maximum pressures of 3000 kg./cm.². Various inhibitory effects were observed, including (1) partial loss of motility, (2) decrease in the ability to multiply, (3) various changes in metabolism, and (4) a decrease in virulence. Many forms were studied, and it was noted that *B. anthracis* and yeast were particularly resistant to the effects of pressure. Further, Chlopin & Tammann reported that the effect of a constant pressure was proportional to the time it was allowed to act and that it was greater at higher temperatures. They also believed that a greater effect was produced when the pressure was applied suddenly.

The foregoing work has been extended by investigations from three different laboratories, employing much higher pressures, the results of which are in essential agreement. Of these the first and most extensive series of experiments is that of Hite, Giddings & Weakley (1914) made at the West Virginia Agricultural Experiment Station. Maximum pressures of 150,000 lb./in.² (over 10,000 atmospheres) were obtained. On cultures of *B. prodigiosus*, *B. fluorescens liquefaciens*, *B. lactic aerogenes*, and *Streptococcus lacticus* the pressure death-point was determined to be as follows: for an exposure of about 4 min. pressures between 85,000 and 100,000 lb./in.² were required; for a 10-min. exposure 50,000–65,000 lb.; and for an exposure of 1 hour sterilization of the culture occurred between 30,000 and 45,000 lb. Certain modifications of the culture medium resulted in a lower pressure death-point. *B. typhosus* and *B. diphtheriae* were slightly more sensitive to high pressures, being killed by 10 min. at 40,000–45,000 lb. *B. subtilis*, in young cultures to avoid spore forms, required somewhat higher pressures for their destruction. A number of experiments were carried out on yeast (*Saccharomyces cerevisiae* and *S. albicans*), the pressure death-point of which was found to be for a 5-min. exposure about 85,000 lb.; 10-min. 55,000–60,000 lb.; and for 1 hour 30,000–35,000 lb. Hite, Giddings & Weakley were also successful in the application of pressure in the preservation of milk and canned goods. In the case of milk, however, slow changes continued after pressure treatment, thought to be due to enzymes which escaped destruction. In all instances in which a medium was sterilized by pressure it remained so indefinitely, as shown by the failure to obtain growth on culturing weeks or months later.

In a study directed toward the possible separation of antigens from pathogenetic organisms Larson, Hartzell & Diehl (1918) studied the effects of pressures up to 12,000 atmospheres on *B. typhosus*, *B. coli*, *B. tuberculosis*, *B. proteus*, *B. subtilis*,

staphylococci, streptococci, and pneumococci. The bacteria were mixed with infusorial earth and the pressure applied directly by means of a piston for a period of 14 hours. Pressures of 3000 atmospheres were not sufficient to destroy any of the bacteria studied, while 6000 atmospheres destroyed all non-spore forming organisms. The spores of *B. subtilis* were much more resistant, not being regularly killed by the highest pressure employed—12,000 atmospheres. The influence of the duration of compression was not studied, and it was thought that the observed effects might, in part, be due to the sudden release of pressure. Filtrates from the pressure treated cultures (6000 atmospheres) had excellent immunizing properties.

In France, Basset & Macheboeuf (1932) have recently employed hydrostatic pressures up to 17,600 atmospheres in relation to their influence on bacteria and various enzymes. All species of bacteria studied retained their capacity to reproduce when subjected to from 3000 to 4000 atmospheres for 45 min. Pressures of 6000 atmospheres killed the non-spore-producing bacteria, *B. prodigiosus* and *Staphylococcus aureus*. Spore-producing organisms, on the other hand, survived compression for 45 min. under the highest pressures employed—17,600 atmospheres, or over 250,000 lb./in.²

It will be noted that the more recent results conform closely to the pioneer studies of Hite, Giddings & Weakley (1914). The latter have the advantage of a large number of observations at various magnitudes and durations of pressure, making possible the construction of time-pressure death curves. As a result of these various observations it appears to be well established that (1) lethal pressures for the non-spore-forming bacteria lie between 3000 and 6000 atmospheres; (2) much higher pressures are required to kill the spore forms; (3) the chief effects occur during the period immediately following the application of pressure, but that at certain critical pressures the time factor is of importance; and (4) the effectiveness of pressures in destroying bacteria may be modified by the culture medium. The part played by temperature requires further investigation as well as the possible influence of the rate of compression and release.

It is probably significant that the magnitude of the pressure required to kill bacteria is of the same order as that producing the first perceptible clouding of protein solutions (see section IV). Since this is in the nature of an irreversible denaturization, it is not unlikely, even in the absence of any precise knowledge of the nature of bacterial structure, that it plays a part in bringing about the death of the organism. Under these circumstances it is of special interest that bacteria in the spore stage are able to withstand enormous pressures, just as they do high temperatures, facts suggesting that proteins of the type occurring in protoplasm generally are not present in bacterial spores. As yet no studies have been reported on the functional activity of bacteria while under compression, experiments which would have special interest in relation to the influence of sublethal degrees of hydrostatic pressure.

On protozoan forms we have the investigations of Regnard (1884 *b, e*, 1891, p. 154), who studied the effects of pressure on a number of Infusoria, including *Paramecium*, *Colpoda*, and *Vorticella*, and emphasized their great resistance to

pressure as compared to higher forms (frogs, fish, etc.). Exposure to 600 atmospheres for 10 min. stopped all ciliary movement, and in the case of *Vorticella* the stalk, which normally assumes a spiral form, becomes much elongated and perfectly straight. Ciliary movement returned within an hour after decompression, and full recovery within 2 hours. In the same year Certes (1884 a, c) made similar studies on the same forms and reported somewhat better survival than was the case in Regnard's experiments. Even after a pressure of 500 atmospheres over a period of 24-48 hours many organisms, including *Chlamydococcus*, *Paramecium*, *Vorticella*, *Euplotes*, and *Pleuronema*, were still alive, but others, including individuals of the same species, were dormant or dead. Marsland & Brown (1936) have recently studied on *Amoeba proteus* and *A. dubia* the effects of rapid compression up to 450 atmospheres applied through the culture medium. Cessation of amoeboid movement occurred when the pressure reached 250 atmospheres, and there was no further effect until a pressure of about 450 atmospheres, when there was a sudden retraction of pseudopodia and the formation of terminal spheres at the distal end of each pseudopodium. On removing the pressure amoeboid motion started immediately, provided the period of compression was not longer than 10 min. Longer compression delayed the return of activity for some minutes, and an exposure of 1 hour was likely to kill the amoeba. When allowed to become equilibrated to the pressure by a 5-min. exposure, the length of the pseudopodia was found to vary with the degree of pressure, viz. there was a lengthening as the pressure was increased up to 136 atmospheres, but thereafter both the length and the diameter were reduced until at about 400 atmospheres no pseudopodia formed and the animal became spherical. These effects are explained on the basis of an increase in the fluidity of the plasmagel and an inhibition of the process which maintains the streaming of plasmasol. Evidence of an increased fluidity under pressure has been obtained by these workers (Marsland, 1934; Brown and Marsland, 1936), using the centrifuge method. The granules of the plasmagel are displaced, giving a hyaline zone, and the time required under standard conditions for this segregation is taken as proportional to the fluidity of the gel. In general the rate of separation was found to bear a direct relationship to the pressure up to 10,000 lb./in.², the effects of given increments of pressure becoming less as the total pressure was increased. Following rapid compression evidence of liquefaction was found to occur in less than a second or so and was rapidly reversed on decompression, suggesting a primary effect on the physical properties of protoplasm. It would be difficult, however, to eliminate completely the possibility that the indicated changes in viscosity produced by the higher pressures are secondary to changes resulting from stimulation or chemical reactions, especially since the general appearance and shape of the centrifuged amoeba are changed with increasing pressures when prolonged. Moreover, Brown & Marsland are careful to point out that measurements obtained from the sedimentation rate of granules do not constitute a valid measure of viscosity in the strictly physical sense because the rate may be modified by changes in protoplasmic activity, interfacial barriers, and other factors.

VII. HIGHER FORMS

The effects of hydrostatic pressure up to 1000 atmospheres has been examined by Regnard (1884 *a, b, e*, 1891) on examples from nearly every phylum of the animal kingdom. The experimental conditions are not presented in as great detail as could be wished, and at times there is doubt as to the duration of compression and especially as to the medium in which the animals were placed. It was always possible to kill the organisms by exposure to a sufficiently high pressure, *i.e.* between 400 and 1000 atmospheres, but some forms were more susceptible than others. Many were inactivated by a pressure of between 400 and 600 atmospheres for 1 hour. In this group are included molluscs (mussels, whelks, tritons, *Cardium*), annelids (leech, *Nereis*, *Serpula*), crustaceans (crayfish, hermit crab, *Gammarus*, *Cyclops*, *Daphnia*), and ascidians. Echinoderms (*Asterias*) and coelenterates (*Alyconium*, *Actinia*) were much more resistant to pressure and survived 1000 atmospheres for 1 hour. Such treatment, however, resulted in great swelling, and normal activity returned only after some hours. In certain experiments (Regnard, 1885 *b*, 1891) with small crustaceans the animals were observed during compression and decompression by means of an optical system which focused an image of the organisms on a screen. Up to 100 atmospheres no effect was noticed, but beyond this there was a gradual loss of activity and the animals settled to the bottom of the container, preceded by tetanic-like movements. The sudden application of 400 atmospheres caused immediate inactivation, and recovery was also prompt if the period of application was short. With each sudden addition of 20 atmospheres' pressure convulsive movements were observed followed by inactivity. Regnard concludes that increased pressures first stimulate and then depress the nervous system. Somewhat similar experiments were made by Hill (1912, p. 116), who reported that leeches and the larvae of gnats were rendered temporarily motionless by a pressure of 500 atmospheres applied for 1 hour and were destroyed by the same exposure to 700–900 atmospheres. Starfish and crabs were killed within 10 min. by 500 atmospheres. "Shell-fish", a "sea-cucumber", and two "sea-worms" survived this pressure.

The vertebrates studied by Regnard included frogs and fish. Because the presence of air causes death by embolism upon decompression it was necessary to remove the air from the lungs or the swim bladder by first exposing the animal to negative pressure. In a fresh-water fish, the "cyprin doré", with emptied swim bladder, 100 atmospheres was without effect, but a reversible loss of motility occurred at 200 and death at 300 atmospheres. At 400 atmospheres the tissues became rigid. Similar results were obtained with small marine flatfish (1884 *d*) having no swim bladder, where a 10-min. exposure to pressure was sufficient to inactivate them for several hours. In frogs (1884 *a*, 1891) the results of pressure were much the same, *viz.* there was no observable effect at 100 atmospheres, which corresponds to the experiments of Hill & Macleod (1902), who directly observed the capillary circulation of frogs while the pressure was raised to 70 atmospheres and saw no change. However, in Regnard's experiments complete loss of excitability and con-

tractility of the muscles occurred at 400 atmospheres, and this was accompanied by the development of an extreme rigidity so that when a force was applied the muscle broke rather than bent. It was also noted that the heart in both fish and frogs was stopped by 600 atmospheres applied for 1 min. The changes in muscle will be further considered in a later section. In this connection it is of interest that tadpoles were quite unaffected by pressures up to 300 atmospheres, and that even at 400 atmospheres the animals recovered in about 4 hours after decompression (Regnard, 1884*b*).

The influence of increased pressure on fish, with particular reference to the metabolism, has been studied by Fontaine (1929*a, b*). On small flatfish (*Pleuronectes platessa*) measurements were made of the oxygen consumption in relation to increased hydrostatic pressures between 25 and 150 kg./cm.² applied for various periods. The maximum increase occurred at between 100 and 125 kg./cm.², and as the pressure was further increased the metabolism fell, the animals being killed at 150 kg./cm.² The oxygen consumption increased with time up to 90 min. and then began to fall. The percentage increases at 100 kg./cm.² for small animals (0.110–0.145 g.) averaged 88 per cent., and for larger ones (0.256–0.300 g.) 74 per cent. These experiments represent one of the few instances in which moderate pressures have been employed for the study of physiological processes and given evidence of an initial stimulating action and thus parallel the effect of pressure on muscle to be described later.

It is of interest to consider the experimentally observed effects of high pressure in relation to the conditions of life in the deep sea. Fish have been captured at depths greater than 2 miles where the pressure is over 300 atmospheres, and many invertebrates exist at much greater depths. These pressures come well within the range producing physiological changes in isolated muscle and death in the higher forms. Thus examples of surface fauna are killed when exposed to pressures comparable to those tolerated by deep-sea species, and conversely the deep-sea forms die when brought to the surface. These facts were the starting-point of Regnard's extensive studies of the influence of high pressure on living organisms. He believed that a sharp distinction was to be made between these two groups, and many of his experiments were directed toward the explanation of this difference. The study is a difficult one, for in bringing a deep-sea animal to the surface not only is the pressure changed but many other environmental factors, such as light, temperature, and the concentration of dissolved gases. The very fascinating problem of the mechanisms involved in the adaptation of the organism to life in the deep sea is one which has not received attention within recent years but seems well worthy of pursuit.

Experiments on the influence of hydrostatic pressure on mammals are, of course, not possible on account of the necessary presence of a gaseous phase. However, there are some observations on the effects of increased air or oxygen pressures which testify to the ability of the higher animals to withstand considerably elevated pressures. Certain observations of the earlier workers in which pressures up to 20 atmospheres were employed have been cited in the introductory section. Hill (1900) found that a pressure of 2 atmospheres was without effect on the blood pressure of cats and dogs, and later with Macleod (1902) observed that the circula-

tion of a hibernating bat was not altered at 20 atmospheres of oxygen. Gaertner (1920) has studied the effects of increased pressures of oxygen, nitrogen, and hydrogen on mice, employing pressures up to 25 atmospheres. Such pressures resulted in no apparent harm to the animals regardless of whether compression took place quickly or slowly. Rapid decompression, however, was fatal—presumably because of the injurious effects of the gases released in the tissues. Such experiments throw no light on the simple influence of pressure, but they are of interest in showing that life, even in the higher forms, is compatible with a considerably elevated pressure.

The maximum depth to which diving operations have been reported, unaided by a rigid suit, is about 300 ft. where a pressure of rather less than 9 atmospheres would be encountered (see Damant, 1930). The limiting factor for human divers is the increased concentration of the respiratory gases which come into equilibrium with the blood and tissues, and it is not probable that the pressure *per se* is accountable for any of the untoward symptoms which may occur.

VIII. DEVELOPMENT

Fifty years ago Regnard (1885*a*) did an experiment with fish eggs (Salmonidae) in which the course of development was followed after exposure for 6 hours to from 100 to 650 atmospheres. The control eggs, as well as those treated with 100 and 200 atmospheres, hatched in 3 weeks. The eggs exposed to 300 atmospheres hatched normally but were delayed 2 days. The 400 and 500 atmosphere eggs were all dead after 6 days, and those treated with 650 atmospheres after 2 days. There have been no further experiments until recently, when Draper & Edwards (1932) investigated the effect of compression on developing ova and on embryonic hearts of the marine fish *Fundulus*, employing pressures between 1200 and 1950 lb./in.² Eggs were placed in the pressure chamber immediately after fertilization and either observed directly through a glass window or they were fixed after being subjected to various durations of pressure. The rate of cell division was compared with that of untreated controls, and it was regularly found that the eggs subjected to pressure developed more slowly. In one series of experiments pressures of 1500–1950 lb., acting for from 1 to 2 hours, retarded the time of the first cleavage about 15 min. The second and third cleavages were also about 15 min. later than the controls, suggesting that the effect was on some process initiating development rather than on the actual mechanism of cell division. In another group of five experiments at 1500 lb. pressure counts were made of the number of eggs in the 2-, 4-, and 8-cell stage with the following results: under pressure the number at each stage was, respectively, 96, 29, and 4; and in the controls, 0, 94, 32; data which again demonstrate a definite retardation in the development of the compressed eggs. Such eggs, when allowed to complete their development, showed a high incidence of abnormalities, such as gross distortions of the body, changes in the cardio-vascular system, and a tendency toward anophthalmus.

In older embryos the heart is slowed during compression and rhythmic activity may be completely suppressed (Draper & Edwards, 1932). The writer (unpublished)

has kept such embryos under 1500 lb. pressure for periods up to 15 hours, during which time the heart remains quiescent and the development of the cardio-vascular system is retarded. Some minutes after the release of pressure normal cardiac action is resumed and the development of the vascular system proceeds normally, but such embryos frequently show developmental abnormalities.

Because of the known effect of pressure in increasing the viscosity of most solutions, and because of the evidence (to be considered later) indicating that there is an increase in the viscosity of muscle during compression, it is not unlikely that the retardation in the rate of cell division under pressure is mediated through an increase in protoplasmic viscosity. However, deductions of this character must be made with caution, and the danger in this instance is illustrated by some recent studies by Brown (1934 *b*) on the changes in "viscosity" in the eggs of *Arbacia* produced by pressure, as indicated by the sedimentation time of the pigment granules in centrifuged eggs. At 23° C. pressures up to 408 atmospheres caused a reversible *decrease* in the viscosity of the cortical zone at any time during the first 33 min. after fertilization, but thereafter was ineffective. The relative decrease in viscosity per unit of pressure decreased as the pressure increased. In the case of unfertilized eggs under the same conditions the viscosity of the interior of the egg, as indicated by the time of formation of the hyaline zone, was unchanged by pressure. Clearly, the pressure-viscosity relationship is not a simple one. Similar procedures applied to amoeba also point to a reduction in viscosity under pressure (Brown & Marsland, 1936).

Mention should be made of Cunningham's (1927, 1934) observation on the effects of increasing atmospheric pressure on incubating hen eggs, although in these experiments the changes observed may well have been due to factors other than that of pressure *per se*. Under pressure (varying between 12 and 15 lb./in.² above atmospheric) there was a marked acceleration of development, especially evident in 3-5 day embryos. Only a few chicks were actually hatched. A failure to develop a normal extra-embryonic circulatory system in a majority of the embryos may have accounted for a high mortality observed at about the 11th day.

IX. BOTANICAL MATERIAL

De Vries, in 1915, subjected seeds of various species of *Oenothera* to hydrostatic pressures between 6 and 8 atmospheres for periods of from 2 to 3 days and thereby got a very much higher percentage of seeds to germinate in comparison with the untreated controls. This effect was explained on the basis of an improved penetration of water through the interstices of the more or less impermeable seed-coat. The effect of high pressure on seed germination has been further studied by Davies (1926, 1928 *a, b*) on *Medicago sativa* and *Melilotus alba*. In these species the percentage germination is normally relatively low, but the application of 2000 atmospheres' hydrostatic pressure for from 5 to 20 min. resulted in a great improvement. In the case of *Medicago* seeds, dried and tested 30 days and 6 months after pressure treatment, an increased germination of over 50 per cent. was shown. In *Melilotus*

similar treatment increased the total germination over 200 per cent. when dried and tested after 30 days, and 150 per cent. after 6 or 10 months. Exposure to 500 atmospheres was less effective even when continued for longer periods. For seeds of *Medicago* exposed to a pressure of 2000 atmospheres it required approximately $2\frac{1}{2}$ times as long to produce a given effect at 0° C. as it did at 20°, while for *Melilotus* the difference was about 5 times.

The mechanism of the pressure action in improving germination is assumed to be a purely physical one. The delay or failure to germinate in the two species studied may be due entirely to the seed-coat, for when the impermeable nature of the coat is destroyed by acids or by mechanical means the seeds germinate readily, but details of the physical changes said to be produced by pressure have not been described. The fact that the pressure influence persists indefinitely favors such an interpretation, but it is not clear why temperature plays such a large role. Another point of interest noted by Davies is that prolonged pressure results in decreased germination, and even at 500 atmospheres it was found that the vitality of the seeds germinating normally might be destroyed, thus more than compensating for the improved permeability of the hard seeds. This is in agreement with some old experiments of Regnard (1884 a), who observed a delay in the germination of cress seeds as a result of exposure to 1000 atmospheres for 10 min.

That chlorophyll continues to function under fairly high compression was observed by Regnard (1884 a, 1891) many years ago. Working on algae, he showed that oxygen formation still occurred under a hydrostatic pressure of 400 atmospheres, although the process was considerably slowed, and algae so treated decomposed after several days. In certain plants, as the potato, false indigo (*Baptisia tinctoria*) and the Indian pipe (*Monotropa uniflora*), blackening is a sign of too rapid oxidation, and Harvey (1922) found that this change could be brought about by exposure to oxygen at a pressure of 100 lb./in.² for 4 hours. However, in hydrogen at between 1300 and 1800 lb. the plants were unchanged for 8 hours (as long as observed), and there was thus no evidence of a pure pressure effect.

X. ISOLATED MUSCLE

Muscle offers a particularly interesting and favorable tissue for the study of the physiological effects of increased hydrostatic pressure for the following reasons: (1) It may be isolated from the rest of the organism and maintained in an approximately normal state in a liquid environment through which the pressure may be transmitted. (2) Its functional activity represents a delicate biological mechanism on which changes in the physical or chemical condition of the cell are certain to be reflected. (3) There are exact methods available for studying both the resting and active processes in muscle. (4) From the standpoint of the investigation of problems relating to muscular contraction the pressure method provides a useful tool in that it may be applied and removed extremely quickly and causes important reversible changes in functional activity.

(1) Physico-chemical factors

(a) *Volume.* From the known effects of pressure on liquid systems a significant decrease in volume of compressed muscle could be safely predicted. The only actual measurements thus far recorded are those of Henderson & Brink (1908), who made accurate measurements of the compressibilities of gelatin solutions and freshly isolated intact muscle of the rabbit. The volume changes were found to correspond closely to those of simple liquid and solid systems, the compressibility becoming less as the concentration of dissolved or colloidal material is increased. The average changes in volume for the several substances studied, caused by 1 atmosphere in the pressure range between 100 and 500 atmospheres, was as follows ($\times 10^{-6}$): muscle 37, 10 per cent. gelatin 39, 0.2 per cent. gelatin 41, water 42. The compressibility of muscle in this pressure range was thus found to be approximately 88 per cent. of that of pure water, and the diminution of its volume under a pressure of 500 atmospheres not quite 2 per cent.

(b) *Length.* In consequence of the smaller volume of muscle under compression some decrease in length is to be expected, although this might be small because of the relatively solid nature of the fibrillar structure as compared to the more fluid sarcoplasm. This has not been directly measured, but in connection with some observations on the visco-elastic changes in isometrically arranged ventricular strips of the terrapin heart and sartorius muscle of the frog (Edwards & Cattell, 1932) it has been found that the equilibrium position following a quick stretch or release showed a greater tension change when the muscle was under pressure, and this denotes a shorter muscle. More recently Edwards (unpublished) has recorded the effect of the addition of weights to isotonicity arranged sartorii and has found that a given increment in weight caused a greater increase in muscle length when under pressure, but that this difference disappeared after the pressure has been maintained for some minutes. Clearly the situation is complex and requires further experiments. Moreover, the interpretation of all these observations is complicated by the fact that there is an undoubted influence of pressure on the elastic properties of the muscle, and it is almost certainly this rather than a direct effect of compression which accounts for the greater part of the observed changes in length. The results may also be modified by the intervention of contractures, which under certain conditions occur at high pressures. Ebbecke's (1914) measurements of the change in length of muscle under pressure represent a measure of this phenomenon rather than that of a decrease in length due to a direct action of pressure.

(c) *Visco-elastic properties.* The experiments described above (Edwards & Cattell, 1932) were made for the purpose of studying the influence of pressure on the visco-elastic properties of muscle. Photographic records of the tension taken during and following a sudden forced increase or decrease in length with and without pressure show three differences due to pressure. The first effect of pressure to be noted is a greater immediate effect on the tension following a stretch or release, *i.e.* the change in length is more completely transmitted to the lever, which denotes a greater muscle rigidity. This might be due to an increase in either elas-

ticity or viscosity or both. Secondly, a quick stretch or release causes a series of highly damped vibrations to be set up in the tension lever, and at the same time there is a gradual adjustment of the average tension to the new muscle length. In the case of a muscle under pressure the damping is greater, and the rate at which the new equilibrium position is reached is appreciably slower. These effects can mean only an increased viscosity, such as has been shown to occur in an active muscle (Gasser & Hill, 1924). The third effect is that described under the last heading, *i.e.* the new equilibrium position. Thus for a given change in length the effect on tension is greater when the muscle is under pressure, a result pointing to an increased elastic coefficient. However, the recent experiments of Edwards (unpublished), showing that for the first few minutes under pressure a given load produces a greater extension and, also, the results of sudden compression and decompression during contraction (see p. 469) make the interpretation of the experiments on muscle elasticity very doubtful.

The evidence for an increased viscosity in compressed muscle accords with expectation on the grounds of the known effects of pressure on the viscosity of solutions. Further, certain of the effects of pressure on muscular contraction (Cattell & Edwards, 1932; Cattell, 1935 *a*) are strongly suggestive of an underlying viscosity increase in the muscle substance. These will be discussed later.

As yet no measurements of the changes in viscosity or elasticity have been made which permit of their expression in exact physical terms. The conditions are not simple on account of the complex nature of protoplasm, especially of muscle, and in the case of viscosity the word is here used in the sense of an increased internal resistance to movement.

(*d*) *Chemistry.* Some of the observations on the changes produced by pressure in intact organisms already recorded have a bearing on cellular metabolism, but practically nothing is known of the effects of compression on isolated muscle. The problem is complicated by the fact that the application of high pressure causes the muscle to go into a reversible contracture which partakes of some of the characteristics of muscular contraction, and the chemical changes which have been described are undoubtedly secondary to this effect. Regnard (1884 *c*, 1886) noted that following compression a muscle or other tissue gained weight and had a swollen appearance, and he believed that the harmful effects were due to the forcing of water into the protoplasm by the pressure through some not clearly defined mechanism. Histological examination by Regnard & Vignal (1884) of muscles exposed to 600 atmospheres for from 10 min. to several hours revealed marked structural disorganization, especially when compression was prolonged. The transverse striation became less marked or nearly disappeared, the bundles of fibrils were separated by a swelling of the sarcoplasm, and the sarcolemma was separated from the rest of the cell. These changes were absent when the muscle was placed in a rubber bag separating it from the water medium, thus lending support to Regnard's hypothesis. It appears, however, that the muscles were always placed in water rather than in an isotonic solution, and this may account in part for the imbibition of water, although these various changes were absent in the controls

without pressure in the same medium. In a hypotonic solution there are several ways in which the rate of water imbibition might be increased by pressure, viz. the chemical changes associated with the production of a contracture, as mentioned above, the fact that the volume change of the muscle would increase the concentration of dissolved materials, and also, changes resulting from a greater hydration of the proteins.

Recent studies by Fontaine (1927 *a*, 1928 *a*) have shown that the swelling described by Regnard is not entirely determined by a hypotonic medium, in that it occurred following compression in muscles immersed in hypertonic solutions. Muscle from the eel and from the hind-quarters of the frog were exposed to pressures of 600 kg./cm.² for 9 min. and 500 kg. for 30 min. respectively, after which the pressure was atmospheric. In the course of the following hours there was a steady gain in weight, whereas the controls continuously lost weight during the same period. A further study (Fontaine, 1928 *b*, 1930) has shown that the changes produced by pressure were similar to those resulting from tetanic stimulation, *i.e.* there was, in addition to the increased weight due to the imbibition of water, an accumulation of lactic acid and a lowering of the pH values. These changes were found to be peculiar to muscle, no such effects having been observed in blood (1927 *b*) and liver tissue (1928 *b*). Ebbecke (1935) has found changes in the content of glycogen, lactic acid, phosphoric acid, and water which parallel the production of contractures in muscle, and Brown (unpublished) has observed an increase of 150 per cent. in lactic acid accompanying pressure contractures at 450 atmospheres lasting about 1 min. While Fontaine reports no observations during actual compression, it is probable that a pressure contracture supervened, and this would explain the changes reported by him.

(2) *Resting muscle*

(*a*) *Lethal action.* That hydrostatic pressure if sufficiently great is injurious to muscle tissue has long been known. Regnard, in 1891, published records of the heart beat of the frog following a short period of exposure to pressures up to 300 atmospheres and found that pressures between 400 and 500 atmospheres, applied to frog's muscle in water, caused a swelling and rupture of the fibres. Essentially similar results have been reported by Hill (1912, p. 115), who observed that at 300 atmospheres for 2 hours there was no diminution in the beat of the frog's heart and no decrease in the reflexes, but that a pressure of 400 atmospheres for 1 hour caused reflex responses to fail, and he also describes structural changes, with some disorganization of the muscle cells and alterations in the myelin of the nerve fibre. However, the experiments of Henderson, Leland & Means (1908) on frog muscle demonstrated the possibility of survival after compression up to 500 atmospheres. Pressure was slowly applied and released over a period of 20 min., after which the isotonic contraction was compared with the control before compression, and it was found, in at least one instance, to be very little changed. Ebbecke (1914) made an extensive study of the effects of hydrostatic pressure on striated muscle (frog) and noted, in the pressure range between 300 and 400 atmospheres, twitches or a

sustained shortening followed by loss of excitability and rigor when the pressure was maintained. Fontaine (1930), from his studies of the effects of high pressure on muscle and other tissues, reached the conclusion that irreversible changes occur in most forms of protoplasm at about 700 kg./cm.²

Neither Fontaine nor Ebbecke has been able to confirm the structural disorganization described by certain earlier workers, and it is difficult to see how a volume change produced by pressure in material as yielding as muscle could cause such injury. They might, however, occur as a result of the hypotonic medium generally employed or be secondary to the chemical changes and the consequent osmotic pressure differences set up between membranes, a factor which has been minimized in the more recent experiments by the use of an isotonic medium. From a theoretical point of view it is of great interest that the irreversible changes produced by pressure are of gradual onset. This fact seems to rule out a direct physical action accompanying the volume change as an immediate cause and puts further emphasis on the secondary reactions involving the breakdown of chemical substances. The magnitude of pressure causing injury to muscle is only a small fraction of that required to precipitate proteins, but protein precipitation is a gross phenomenon and it is not unlikely that the initial effects occur much earlier. Moreover, in speculating upon the mechanism of pressure action it is necessary to take into account the numerous phase boundaries which involve transitions of a very varied chemical and physical nature and which might, therefore, be very differently affected by pressure, with consequent shifting of equilibria. It is probable that in some of these finer relationships, now more or less inaccessible to experimentation, the mechanism of pressure action will be ultimately elucidated.

(b) *Contracture*. A significant discovery was made by Ebbecke (1914) in the observation that the application of hydrostatic pressure may cause a contracture in striated muscle quite apart from any other stimulus. Ebbecke studied this influence of pressure in some detail and showed that the shortening produced was of the nature of a reversible contracture, such as is produced by certain chemicals or strong electric currents. A pressure of 200–300 atmospheres briefly applied to the gastrocnemius muscle of the frog was without effect, but between 300 and 400 atmospheres a twitch occurred with each application of pressure. The same result was obtained in curarized muscle. Greater pressures or those of longer duration resulted in a continuous shortening which showed no fibrillar or “spontaneous” contractions and was characterized by the absence of an accompanying action current on the string galvanometer. A superimposed electrical stimulus gave a normal response and a typical action current. This contracture lasted only during the application of pressure, and it was noted that the shortening was greater in a cold muscle. With higher pressures there was a slower subsidence of the tension, loss in excitability, and permanent injury to the muscle. Thus there is a change produced by pressure which in all respects is typical of the peculiar functional shortening known as a contracture. This involves the liberation of energy and in general is a non-conducted disturbance (see Gasser, 1930). In the case of a pressure contracture there can be no conduction, since the full length of the fibre is equally and

simultaneously affected, and presumably the excitable system of the cell is not involved.

The phenomenon of pressure contracture has been further studied by Brown & Edwards (1932). This contracture may be elicited in striated, smooth, and cardiac muscle, but details have been published only for the striated retractor penis muscle of the turtle. A hydrostatic pressure of 2000 lb./in.² or over at 4° C. gives a slowly developing but prolonged contraction starting immediately. The tension increases to a maximum and, if the pressure is maintained, then subsides at a slow rate, finally returning to the base-line. Sudden decompression results in an initial increase in tension followed by a quick return to zero. The total tension is directly proportional to the degree of pressure and rises along an S-shaped curve, approaching an upper limit, which may amount to 90 per cent. of the tension of a maximum tetanus, at 8000 lb. pressure. If a muscle is stimulated while in a state of pressure contracture, a normal twitch results superimposed on the background of activity. Preparations vary markedly in the degree of pressure contracture under apparently similar conditions. The best results were obtained with the muscle immersed in blood serum at low temperatures. With repeated applications of pressure the response was gradually reduced, but recovery took place after a few hours' rest.

The fact that the contracture tension is abruptly terminated upon decompression has recently been utilized by Brown (1935 *a*) to gain evidence on the time course of energy conversion. By decompressing at various times during the development of contracture it is possible to ascertain the rate of energy release and compare it with that of a normally excited muscle. Experiments performed in this way show that if the muscle is decompressed when only 5 per cent. of the expected contracture tension has had time to develop, the subsequent contraction resembles in rate and form a simple isometric twitch. Since there is no evidence of an accompanying propagated action potential, it is believed that during the brief period of compression a quantity of energy is liberated by a direct action of the pressure which, after decompression, causes contraction in the normal way.

(3) *Muscular contraction*

(*a*) *Augmentation*. It was discovered in 1927 (Edwards & Cattell, 1927, 1928) that the application of hydrostatic pressure to cardiac muscle profoundly modifies contraction, the force of the response or degree of shortening being markedly increased. A more exact analysis of the pressure-energy relationship was later made on striated muscle (Cattell & Edwards, 1928), using the gastrocnemius from the frog and measuring the tension by means of an optically recording isometric lever and the initial heat production with thermopile and galvanometer. At that time apparatus was available for studying the effects of pressures up to only 1000 lb./in.² For a single maximal twitch this degree of pressure caused an average increase of about one-third in the tension developed, and for intermediate grades of pressure the augmentation was approximately proportional. The effect probably occurs *pari passu* with the pressure change, and certainly very promptly. This is shown by experiments (Cattell & Edwards, 1928) in which pressure has been applied

suddenly to a muscle contracting at the rate of several per second or during the latent period (Brown, 1936 *a*), when, in both instances, the succeeding response is fully augmented.

The initial heat production of the compressed muscle showed an increase at all grades of pressure corresponding with the increased tension, so that the efficiency of the process as measured by the Tl/H relationship was unchanged. These observations are significant in showing that the mechanism of the pressure effect involves an increase in the amount of chemical potential energy set free rather than a shift toward a greater efficiency in the utilization of that energy. In contrast to the case of a twitch no obvious effect of pressure was observed for a tetanic stimulus. This difference was discussed by the authors in relation to current theories of muscular contraction, and it was shown to be compatible with the suggestion of Hartree & Hill (1921) relating to the control of energy for contraction.

Further experiments of this type have been made employing the sartorius muscle of the frog and higher pressures (Cattell & Edwards, 1932). Above 1000 lb./in.² the augmentation in tension per unit increase in pressure becomes gradually less and a maximum effect is reached between 2000 and 4000 lb. at room temperature. As the pressure is further increased the twitch becomes gradually less and is prolonged, as discussed later. In the group of experiments just referred to the maximum tension was increased to between 20 and 42 per cent. of the atmospheric pressure values. The effect on cardiac muscle is even greater, a several-fold increase occurring at the optimum pressure. The effect of pressure in increasing the energy liberated during a contraction has also been demonstrated by Brown (1934 *a*) in the retractor penis muscle of the turtle by quick decompression at various times during the contraction cycle. An analysis of these results, given in detail later, confirms the conclusion that the augmentation in tension in the twitch under pressure is due to an increase in the quantity of the tension-producing substance.

It is of interest to consider the stimulating action of hydrostatic pressure in relation to the discovery of Ernst (1925) that a contracting muscle undergoes a decrease in volume. Further studies (1927) have shown that the changes parallel the action potential and thus are associated with the early effects during the period of excitation rather than with the later mechanical events. (For further literature on the subject see Ernst & Koczás, 1935). Meyerhof and his associates (Meyerhof & Möhle, 1933; Hartmann, 1934) observed in addition slower changes outlasting a short tetanus which they believe are to be explained by the underlying chemical reactions. They measured the volume changes in a number of the reactions known to be associated with the process of contraction and found, for example, that the pyrophosphate and phosphocreatin hydrolysis resulted in a smaller volume. It is attractive to consider these observations in connection with the effects of pressure, but at the present time they appear inadequate to explain them. Pressure might be expected to shift the equilibrium point through a differential influence on the volume of the reacting substances, but it is not clear how this could explain an effect on the velocity or magnitude of the chemical reactions.

(*b*) *Changes in fatigue.* As has been indicated, there is great variability both in

the magnitude of the augmentation and in the optimal pressure for its production. At certain seasons of the year or at least in different batches of frogs marked variations occur, and no constancy is observed from experiment to experiment. This contrasts with the results obtained from any one preparation in which reproducible results may be obtained over and over again with the repeated application of pressure. The conditions responsible for the natural variability are not known, but some of the factors which influence the magnitude of the pressure augmentation have been studied. A moderate degree of pressure is not directly responsible for the change, for the full effect may still be present after several hours of compression. On the other hand, if the muscle is fatigued or is in poor condition due to overstimulation or otherwise the pressure influence is completely altered, viz. the same pressure, which earlier had caused an augmentation in the response, now has an opposite effect, *i.e.* becomes smaller than is the case at atmospheric pressure, just as occurs in fresh muscle at much higher pressures. It is probable that the phenomenon of pressure reversal just described is brought about, at least in part, through an effect of lowered pH on the viscosity of protoplasm (see Cattell & Edwards, 1932, p. 32).

(c) *Role of temperature.* A second factor having an important influence on the pressure effect is that of temperature. This was first observed for cardiac muscle (Cattell & Edwards, 1930). The tension of the muscle twitch has a negative temperature coefficient, becoming greater as the temperature is lowered in the range between room temperature and about 5° C. (Doi, 1920). In the case of moderate temperatures and pressures there is a summation of the separate effects of each and a still greater tension results. It was suggested that the parallelism between the effects of low temperature and high pressure on physical systems, both of which tend to constrain the freedom of molecular motion, might be the basis for the similarity of their effects on muscular contraction. If either of these factors is pushed too far the augmentation fails to occur, and it was shown that at about 5° C. pressure causes no improvement in the response. On striated muscle the influence of temperature in modifying the pressure effect is similar (Cattell & Edwards, 1932). As the muscle is cooled the augmentation produced by an optimum pressure becomes less until finally, usually between 9 and 14° C., the sign of the effect is reversed, *i.e.* the application of pressure results in a smaller twitch tension than the atmospheric pressure control. When the temperature is sufficiently reduced an increased pressure causes the response to become very small or completely obliterated. With higher pressures the power to develop tension in response to a single shock of whatever intensity is completely lost. The response to a tetanic stimulus is retained to a greater degree. The changes produced by temperature and pressure are completely reversible.

(d) *Contraction time.* A second effect of pressure is to be noted in the time relations of the twitch. In the case of cardiac muscle (Edwards & Cattell, 1930) moderate pressures causing a considerable increase in tension had comparatively little effect on the duration, especially the phase of shortening. In one group of eighteen experiments, made on ventricular muscle of the turtle subjected to a pressure of approximately 1000 lb./in.², the tension increased in the average 42 per cent.,

the phase of contraction 1.5 per cent., and the phase of relaxation 9.5 per cent. Under 1500 lb. the augmentation was 68 per cent., and the phases of contraction and relaxation were lengthened, respectively, 8.0 and 15.5 per cent. In the case of striated muscle the effects of pressure are similar, viz. there is very little effect up to between 3000 and 4000 lb. in the range causing an augmentation, but with higher pressures which depress the response there is a several-fold increase in the duration of the twitch. At low temperatures and in fatigued muscle the slowing of contraction and relaxation occurs at much lower pressures, corresponding to the failure to augment the twitch tension.

(e) *Efficiency.* The foregoing illustrates three conditions in which pressure causes a depression in the tension of the twitch—cold, fatigue, and higher pressures—and in all of them there occurs also a prolongation of the contraction-relaxation period. There is reason to believe that high pressure, low temperature, and increased acidity all tend to produce an increase in the viscosity of protoplasm.¹ Indeed, as already described (p. 459) quick stretch and release experiments on muscle give evidence of an increased viscosity under compression. Furthermore, there is much evidence to indicate that the external form of the tension curve of a twitch, due to a delay in overcoming the viscous resistance, does not represent the immediate expression of the time course of energy expenditure.² Thus any increase in the resistance to internal movement would be reflected in a smaller and slower response. If this is the mechanism through which the higher pressures bring about a depression of the response there must necessarily be some loss in efficiency due to the extra energy required to overcome the increased internal resistance.

Recently experiments have been carried out in which simultaneous measurements were made of the initial heat production and tension in the twitch of the frog's sartorius muscle with and without pressure (Cattell, 1935 *a*). The experiments were carried out at a low temperature (6–9° C.) in order that a depression in the response might be obtained at conveniently low pressures. Without exception there was a fairly large increase in the heat production when the muscle was under pressure, an effect which was greater than that on the tension, thus showing a fall in efficiency as measured by the tension-heat ratio. In many experiments a depression in the tension of the twitch was accompanied by an increased heat production, and the maximum loss in efficiency was usually between 20 and 30 per cent. In general this change is a reversible one and can be repeated a number of times. Earlier experiments by Brown (1934 *a*), in which the muscle (retractor penis of turtle) was suddenly decompressed during contraction, resulted in an immediate increment in tension and this almost certainly denotes an improved efficiency. Brown (1936) has extended the study of the changes associated with compression and decompression to cardiac muscle and attributes the changes in efficiency to effects on elastic factors.

¹ For a full discussion of the evidence bearing on this point see Cattell & Edwards (1932).

² The recent experiments of Fenn & Marsh (1935) and of Brown (1935*a*) may necessitate a modification of this view.

Higher pressures result in a decrease in both tension and heat without much further effect on efficiency until finally no response can be obtained. The latter effect comes on gradually while the muscle is under the higher grades of compression and not *pari passu* with the pressure increase. This has been demonstrated by the sudden application of such pressures to a muscle responding to single shocks at the rate of several per second when the first few responses following are fully augmented, but successive responses decline at first very rapidly and later slowly (Cattell, 1935 *a*). Further, Brown (1936), by the sudden application of high pressure to cardiac muscle, has shown that an augmentation results only when the stimulus is given immediately following the application of pressure but completely fails after the lapse of a short period of time. When the response is obliterated by pressures up to at least 15,000 lb./in.² complete recovery occurs, provided the application has been of short duration. The question arises as to whether the decline in the response represents a failure of part of the fibres to contract or to a loss in power of all the fibres. The latter is almost certainly the correct explanation, since the response cannot be improved by increasing the strength of the stimulus and the same phenomenon occurs in cardiac muscle without evidence of a failure in conduction.

(*f*) *Résumé.* The experiments just described give evidence of three distinct and probably independent effects of increased hydrostatic pressure on muscular contraction. As the pressure is increased the first change to occur is the conversion of more energy, which is shown by an augmentation of the twitch tension and a corresponding increase in the initial heat production. Next there is evidence of an increased viscosity and decreased elasticity, and these factors adequately explain the smaller and slower twitch as well as the reduction in efficiency. Finally there is a gradual loss in the ability to contract under the higher pressures, which in the beginning is reversible, but if long continued results in permanent loss of function. As already described, the pressure itself causes a development of tension or contracture, and this may be in part responsible for the falling off in twitch tension during the prolonged application of higher pressures.

XI. PROPERTIES OF CARDIAC MUSCLE

(*a*) *Comparison with striated muscle.* All the physiological effects of high pressure described for striated muscle can be observed in cardiac muscle, and such differences as have been recorded are of degree only. Much of the experimental data has been given in connection with the discussion of pressure effects on striated muscle. The augmentation consequent upon a given pressure increase is considerably greater, and reversal (depression of the response under pressure) requires a higher pressure and lower temperature for its production, consequently the magnitude of the tension response may be pushed much farther. At room temperature the maximum contraction occurs at about 6000 lb./in.² when it may be from 4 to 6 times that at atmospheric pressure. The effects of pressure are far more uniform and thus fatigue and low temperature must be more extreme in order to bring about

a reversal in the pressure augmentation. It has been suggested that the reason for these differences may be the much slower contraction process in cardiac muscle, and thus viscosity would play a smaller role and the changes produced by pressure on this factor would be less important (Cattell & Edwards, 1932). In a rhythmically beating auricular strip pressure produced an immediate augmentation which under 60 atmospheres continued to increase slightly for the following few beats (Edwards & Cattell, 1928). The response was well maintained during compression, but upon release of the pressure it dropped to perhaps 50 per cent. of the pre-pressure value and only gradually recovered over a period of from 5 to 15 min. The general results described above have been confirmed by Brown (1934 *d*), who has made a quantitative study of the pressure-tension-temperature relationship in auricular muscle of the turtle. The pressure-tension coefficient, *i.e.* the unit change in tension per atmosphere of pressure (68–400 atmospheres) was found to be zero from 5 to 8° C.; at temperatures above 10° C. the value was positive and increased with the temperature up to 20° C.; while below 5° C. the value was negative and increased as the temperature was decreased.

In the case of cardiac muscle marked augmentation in contraction is brought about by low temperature or the application of epinephrin. Compression of the tissue under these conditions results in further augmentation, *i.e.* the effect of low temperature or epinephrin is summated with that of the increased pressure (Cattell & Edwards, 1930, 1931). In the case of epinephrin there was evidence of a synergistic effect in that the combined action of this substance and pressure gave a greater percentage increase in tension than that of the sum of the two agents acting separately.

(*b*) *Conductivity.* Evidence of increased conductivity as a result of moderate compression was observed in some experiments on whole frog hearts showing impaired conduction between auricle and ventricle (Edwards & Cattell, 1928). In one instance, in which the heart was showing a two to one block, normal rhythm was restored by the application of 60 atmospheres, with recurrence upon decompression, and this was repeated a number of times. The pressure augmentation is always accompanied by an increase in the rate of rhythmically contracting muscle, *e.g.* in eighteen experiments at 60–80 atmospheres the average duration of the heart cycle was decreased from 2.46 to 2.27 sec. This quickening was most marked when pressure was first applied and in many gradually subsided after some minutes of compression, although the augmentation persisted. The rate, as well as the tension, for a time after pressure release was always less than the pre-pressure value.

(*c*) *Refractory period.* Measurements of the least interval between two stimuli causing double responses in auricular and ventricular muscle of the turtle heart have been made, using a Lucas pendulum (Cattell & Edwards, 1929). A pressure of 1000 lb./in.² prolonged the refractory period on the average 5.4 per cent. in seventeen ventricular preparations. In six experiments with auricular muscle subjected to a pressure of 1550 lb./in.², a pressure causing an increase of over 100 per cent. in the tension developed, the average prolongation of the refractory period was 17 per cent. It was suggested that these results may not represent a specific

influence on the recovery rate but that the prolongation of the refractory period is a consequence of the augmented contraction, following which more time is required for restoration. The possibility that the determination of the refractory period was influenced by an increased resistance in the stimulating circuit resulting from the pressure was not ruled out in these experiments, although such an influence would be minimized by the very strong stimuli employed. Later measurements of the chronaxie of cardiac muscle (Fredericq & Cattell, unpublished) gave no evidence of a consistent effect of pressures up to 1600 lb./in.² on either the rheobase or chronaxie.

(d) *Action potential.* Using small strips of auricular muscle of the terrapin heart stimulated by the natural sinus rhythm, Edwards & Brown (1934) have made a study of the action potential in relation to various degrees of compression. Pressures up to 5000 lb./in.² increased the amplitude of the initial spike, as recorded on the string galvanometer, and also increased slightly the maximum voltage of a following sustained plateau-like component. These changes closely followed the accompanying increase in tension. As the pressure was increased above 6000 lb. the spike became smaller and slower, the plateau phase abbreviated, and finally the monophasic action potential became diphasic. Very high pressure (12,000–15,000 lb./in.²), which abolished the mechanical response, also obliterated the rhythmic potentials or reduced them to small waves which then had the appearance of non-conducted disturbances originating in the sinus. The changes were reversed upon release of the pressure, provided its application was not too long nor frequent. It was deduced that pressures below about 5000 lb. increase excitability and above this level inhibit rhythmicity, and also decrease conductivity, thus limiting the region of activity and bringing about a diphasic spike.

XII. SMOOTH MUSCLE

The only published description on the effects of increased hydrostatic pressure on smooth muscle is that of Edwards (1935). On small strips from the pyloric end of the stomach of the "painted" terrapin it was found that the tension response to a 10 sec. faradic stimulus in seventeen experiments was reduced 54 per cent. on the average by the application of pressures ranging from 200 to 1500 lb./in.² This effect was completely reversible and actually the first response following decompression was frequently greater than the control taken before the application of pressure. At higher pressure a contracture was produced, just as in other types of muscle. The tension developed during such a contracture produced by a pressure of 5000 lb. was sometimes greater than could be obtained from a maximal tetanic stimulus. In collaboration with Dr J. W. Draper the writer (unpublished) has made observations on rhythmically beating strips of muscle from the pylorus and the duodenum. To increase the rhythmicity a small amount of pilocarpin was added to the Ringer's solution in which the muscle was immersed. Under these conditions the rhythmic activity of the pyloric strips was momentarily stopped by the application of pressure (1200–1300 lb./in.²), and on recovery the response was usually smaller and the rate slower than was the case in the pre-pressure record. Upon release there was

evidence of an increased excitability in a very large and often long, irregularly sustained, contraction. In the case of intestinal strips such pressures always resulted in a complete cessation of spontaneous activity and the muscle remained in a condition of increased tonus, the tension being about half of that at the peak of the previous rhythmic response. As with the pyloric strips, a single prolonged contraction occurred coincident with decompression, following which the normal rhythm was resumed. Contrasting with the results described above, some experiments made by Brown (1935 *b*) on smooth muscle from the mantle of the sea-slug, *Alphysia protea*, gave an increased contraction at pressures from 68 to 200 atmospheres.

Clearly the picture of the pressure effect on smooth muscle is a complicated one and requires further investigation. It differs in an interesting way from striated muscle in that evidence of an improved contractility under pressure has not usually been observed. It is possible that the mode of stimulation employed (tetanus) and the absence of an all-or-none response may play a part. Viscosity is generally believed to play a more important role in smooth muscle and it may be that the pressure influence on this factor masks any stimulation of the underlying processes of contraction. In this connection measurements of the heat production would be of interest.

XIII. QUICK APPLICATION AND RELEASE OF PRESSURE

An important application of the pressure method has been made by Brown by the procedure of quick compression or release during various phases of the contraction cycle. These experiments have thrown light not only on the mechanism of pressure action but also on the time relations of the processes normally concerned in muscular contraction. In the rapid compression experiments (1936) a pressure of 470 atmospheres was applied to the muscle in less than 0.05 sec., either just prior to or during the contraction cycle. In the rapid decompression experiments (1934*a*, 1936) the procedure was similar. The muscles employed were the striated retractor penis and auricular muscles of the turtle and the sartorius of the frog.

The results from compression and decompression agree in showing that pressure applied early in the contraction cycle augments the tension, while later in the cycle it causes depression. The sudden application of pressure after the tension has developed results in an abrupt drop in the tension level, while decompression brings about an equally abrupt rise.

These observations give clear evidence of two distinct effects of pressure. In the first place, as was shown earlier, there is an increase in the mobilization of energy, and the effective period during which this may be brought about is now shown to comprise not more than the initial one-tenth of the contraction period. The process effected at this time is intimately associated with the excitation process, as evidenced by an increase in the spike potential whenever more energy is mobilized and also by the coincidence of the effective period and the duration of the action current.

The second effect of pressure, which is observed as a depression in the tension, is presumably upon the contractile mechanism *per se*. This is interpreted as signifying a decrease in the efficiency with which a given concentration of the contraction producing substance maintains tension and is attributed to a decrease in the coefficient of elasticity of contracting protein elements. In this connection measurements of the heat production would be useful. The abrupt drop in tension following a compression at the peak of the contraction cycle is explainable on the basis of a decrease in elasticity and there is no reason to suppose that it could be mediated by viscous changes. In view of this evidence Brown believes that at high pressure the reduction in efficiency is to be accounted for on the basis of a decrease in the elastic coefficient or a change in the rate of energy conversion rather than to an increase in viscosity.

XIV. NERVE

Regnard (1887 *b*, 1891) attempted some experiments on the effects of pressure on nerve, but their interpretation is made uncertain because apparently the nerve was immersed in water and its function was not tested until after removal from the pressure. After a 10-min. exposure to pressures between 200 and 600 atmospheres the nerve always had a swollen appearance and was increased in weight. Histological examination showed a general structural disorganization, with a separation of the myelin from the neurilemma and an increased prominence of the nodes. The excitability of the nerve was diminished and conduction time plus muscle latency was approximately doubled. In some recent experiments (Grundfest & Cattell, 1935) action potentials were recorded by means of a cathode-ray oscillograph in the frog's sciatic nerve while under compression in a medium of paraffin oil. Under moderate pressures (5000–8000 lb./in.²) a single shock commonly set up more than one impulse in the faster conducting fibres, giving a well-marked elevation at the end of the conducted *A* wave about 2 milliseconds from its start. Such repetitive responses have also been seen by Ebbecke in compressed nerve and were described at the 1935 meeting of the International Physiological Congress. In single fibre responses (Grundfest & Cattell, 1935) a series of three or more impulses might result from a single shock and were probably not related to the intensity of the stimulus, since they occurred when at threshold. These potentials were increased about 10 per cent. and the duration 20 per cent. under pressure and the absolutely and relatively refractory periods prolonged. Thus the influence of pressure on the spike height, the conductivity, and the refractory period correspond with the findings on cardiac muscle. Preliminary observations (unpublished) show that the after-potentials are modified by pressure, especially the positive potential which comes on early and is greatly increased in magnitude.

With higher pressures (8000–15,000 lb./in.²) the action potentials become smaller and the conduction rate markedly slowed. The response entirely disappeared at the higher pressure levels, but all the changes described were completely reversible with decompression. The threshold of the nerve was lowered when the pressure was first applied and then slowly rose until very strong shocks were necessary to excite.

There was a further rise in threshold upon releasing the pressure and this required several minutes before returning to normal values. During these changes in excitability there was no significant change in chronaxie.

These studies point to a primary stimulating action of pressure on nerve, just as has been observed in relation to muscular contraction. At higher pressure this is changed to a reversible depression, but pressure if too high or prolonged completely suppresses normal function and finally kills the nerve fibre, as it does all living cells.

XV. SUMMARY

Our knowledge of physiological effects of pressure is as yet too incomplete to permit of generalizations, and we are still very much in the dark regarding the mechanism of pressure action. Any influence of hydrostatic pressure must be secondary to a decrease in volume. In non-living systems this results in important changes in physical and chemical properties and gives a basis for the explanation of the changes produced in living material. Such factors as the velocity of chemical reaction and the viscosity of fluids are, in general, increased quite out of proportion to the volume change. High pressures bring about an irreversible polymerization of many substances, including proteins. The same order of pressure inactivates most enzyme solutions, bacterial toxins, antibodies, viruses, and other biological agents. The simpler forms of life, such as bacteria and yeast cells, are only slightly less resistant, but all are killed by sufficiently high pressure. On higher forms relatively low pressures cause death, especially when long continued.

There are many known instances where small pressures cause a stimulation of physiological processes, and this may prove to be a general phenomenon occurring at pressures below those resulting in depression. In the case of muscle contracting under pressure there is a marked augmentation in the response, and the application of pressure may, independently of any other form of stimulus, result in the prolonged liberation of energy. Provided that the pressure increase is not too extreme, all the changes observed are reversed with decompression.

The action of pressure on physiological mechanisms has a special interest because it is a fundamental one on molecular relationships extending throughout the cell structure. Furthermore, it is an agent which can be supplied and removed with great rapidity and as such provides a unique tool for the study of physiological problems.

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- (1891). *Recherches expérimentales sur les conditions physiques de la vie dans les eaux*. Paris: Masson.
- REGNARD, P. & VIGNAL, W. (1884). "Des lésions que produisent sur les tissus animaux les hautes pressions." *C.R. Soc. Biol.*, Paris, **36**, 403.
- STANLEY, W. M. (1935). "Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus." *Science*, **81**, 644.

ADDENDUM

Since the completion of the manuscript of this review there have appeared a number of additional papers from the laboratories of Dr Ebbecke, in Bonn, and Drs Basset and Macheboeuf, in Paris. These will be briefly characterized.

EBBECKE, U. & HASENBRING, O. (1935). "Über die Kompressionsverkürzung des Muskels bei Einwirkung hoher Drucke." *Pflüg. Arch. ges. Physiol.* **236**, 405. A detailed study of contractures in striated muscle produced by increased hydrostatic pressure up to 800 atmospheres, using a method which permitted shortening. It is shown that a certain minimal pressure (usually between 200 and 300 atmospheres) is necessary to cause the muscle to contract, and thereafter equal increments of pressure produce proportional shortening. At pressures below about 500 atmospheres immediate relaxation occurred on decompression, but higher pressure resulted in a contraction remainder and, if too high or prolonged, in a permanent loss of excitability. Essentially a confirmation of the earlier work of Ebbecke (1914) and Brown & Edwards (1932).

EBBECKE, U. (1935). "Über die Wirkung hoher Drucke auf Herzschlag und Elektrokardiogramm." *Pflüg. Arch. ges. Physiol.* **236**, 416. On the isolated frog heart pressures up to 400 atmospheres caused an increased amplitude and frequency of the beat, improved conductivity, and, in instances where the heart had stopped beating, a revival of rhythmic contractions. Higher pressures resulted in a depression and finally failure of contraction. These results are in complete accord with those obtained in the Cornell laboratory (Edwards & Cattell, 1928 and later). In the case of a shrimp (*Pandalus*) with the heart *in situ*, increased pressure always caused a slowing. Observations were also made on the effect of pressure on the electrocardiogram of the isolated frog heart, with results very similar to those described by Edwards & Brown (1934) for strips of auricular muscle from the turtle.

EBBECKE, U. (1935). "Über die Wirkungen hoher Drucke auf marine Lebewesen." *Pflüg. Arch. ges. Physiol.* **236**, 648. A study of the effects of pressure applied through oil on the activity of a number of marine forms, including examples of the following classes: Hydrozoa, Actinozoa, Ctenophora, Echinoidea, Crustacea, Arachnida, Pisces. Many species showed evidence of increased activity at moderate pressures. Higher pressures resulted in the cessation of all movement, which was resumed immediately upon release if the pressure was not too great; otherwise a prolonged period of paralysis resulted, as observed by Regnard (1891). In general, pressure affected spontaneous activity in a manner closely analogous to the changes observed in the isolated frog heart.

EBBECKE, U. (1935). "Das Verhalten von Paramecien unter der Einwirkung hohen Druckes." *Pflüg. Arch. ges. Physiol.* **236**, 658. Paramecia, when subjected to hydrostatic pressure, showed diminished speed, and in some cases abolition of movement, increased thigmotaxis, a tendency to rolling movements, and strong geotaxis, effects which were immediately and completely reversed upon release of pressure. (The highest pressure employed was 800 atmospheres.)

EBBECKE, U. (1935). "Kompressionsverkürzung und idiomuskuläre Kontraktion und die Beziehung zwischen elektrischer und mechanischer Reizung." *Pflüg. Arch. ges. Physiol.* **236**, 662. A discussion of pressure and electrical contractures of striated muscle in comparison with the response resulting from a normal stimulus. In all their characteristics electrical and pressure contractures are found to be similar, but quite different from the normal contraction. Two points not previously discussed in this review may be mentioned here: (1) the magnitude of the contracture is the same regardless of whether the pressure is applied slowly or suddenly; and (2) the pressure contracture may still be obtained in muscle made inexcitable by isotonic glucose or narcotics (Ebbecke, 1914).

EBBECKE, U. (1935). "Muskelszuckung und Tetanus unter dem Einfluss der Kompression durch hohe Drucke." *Pflüg. Arch. ges. Physiol.* **236**, 669. On the gastrocnemius muscle of the frog and isotonic lever it was observed that increased pressure results in (1) an increased twitch response, (2) a slowing especially of the relaxation phase, (3) a depression in the response and a contraction remainder at pressures above 500 atmospheres, (4) a decrease in

excitability, and (5) the absence of augmentation in a tetanic response. All these changes were reversed upon decompression. They thus confirm the earlier work carried out in the Cornell laboratory.

EBBECKE, U. & SCHAEFER, H. (1935). "Über den Einfluss hoher Drucke auf den Aktionsstrom von Muskeln und Nerven." *Pflüg. Arch. ges. Physiol.* **236**, 678. The effect of pressure on the action potential of nerve and muscle was studied by means of a cathode-ray oscillograph. In nerve, high pressure caused spreading and reduction in the height of the action potential, an increase in the negative after-potential, and an increase in excitability. In general, the results agree with those of Grundfest and Cattell (1935).

BASSET, J., MACHEBOEUF, M. & PEREZ, J.-J. (1935). "Études sur les effets biologiques des ultra-pressions. Modification de la spécificité antigénique des sérums sous l'influence des pressions très élevées." *C. R. Acad. Sci., Paris*, **200**, 496. Guinea-pigs sensitized to horse serum no longer react when the serum is previously exposed to pressures above 4000 atmospheres for 30 min. However, animals may be sensitized with pressure-treated horse serum (4500 atmospheres, 30 min.), in which case they do not react to untreated serum. Since pressure has no noticeable effect on the acid and base groups of the protein, it is concluded that specificity probably depends on the arrangement of the protein chains in the molecule.

BASSET, J., WOLLMAN, E., WOLLMAN, E. & MACHEBOEUF, M.-A. (1935). "Études sur les effets biologiques des ultra-pressions; action des pressions très élevées sur les bactériophages des spores et sur les autolysines." *C. R. Acad. Sci., Paris*, **200**, 1072. Cultures of *B. subtilis*, contaminated with the corresponding bacteriophage, were subjected to pressures between 10,000 and 13,500 atmospheres. This had no effect on the activity of the bacteriophage, whereas in the absence of its spores the phage is completely inactivated by a pressure of 7500 atmospheres. The effects were thus analogous to those produced by heat.

BASSET, J., WOLLMAN, E., MACHEBOEUF, M.-A. & BARDACH, M. (1935). "Études sur les effets biologiques des ultra-pressions: action des pressions élevées sur les tumeurs." *C. R. Acad. Sci., Paris*, **200**, 1247. The chicken sarcoma of Rous was inactivated by a pressure of 1800 atmospheres but was unaffected by 1000 atmospheres. Sarcoma transplants in rats were completely inactivated by 1800 atmospheres, which was the lowest pressure employed. It is stated that the sensitivity of rat tumors to pressure is greater than any other biological material studied, presumably because the functions of intact cells are involved.

BASSET, J., NICOLAU, S. & MACHEBOEUF, M.-A. (1935). "L'action de l'ultrapression sur l'activité pathogène de quelques virus." *C. R. Acad. Sci., Paris*, **200**, 1882. All the viruses studied were unaffected by treatment for 30 min. with pressures up to 2000 atmospheres. The virus of herpes (in rabbits) was inactivated at 3000 atmospheres. Aphtha virus (in guinea-pigs) was attenuated at 3000 atmospheres and avirulent at 4000. Rabies virus (in rabbits) was unaffected by treatment at 3000 atmospheres, attenuated at 4000, and killed at 5000 atmospheres. Yellow fever virus (in *Macacus rhesus*) slightly attenuated at 3000 atmospheres. Virus of enzoötic encephalomyelitis (in rabbits) was resistant to 6000 atmospheres but inactivated by 7000 atmospheres. It is concluded from these results that the viruses are distinct from enzymes and toxins whose properties survive even 10,000 atmospheres. Further, the resistance of these viruses was not related to their corpuscular dimensions.

Further papers in the field of the physiological effects of pressure have appeared as follows:

BROWN, D. E. S. (1936). "The effect of rapid compression upon events in the isometric contraction of skeletal muscle." *J. cell. comp. Physiol.* **8**, 141.

EBBECKE, U. (1936). "Einwirkung hoher Drucke auf glattemuskulige Organe (Froschmagenpräparat)." *Pflüg. Arch. ges. Physiol.* **237**, 771.

EBBECKE, U. (1936). "Über das Verhalten des Zentralnervensystems (Rückenmarksfrosch) unter der Einwirkung hoher Drucke." *Pflüg. Arch. ges. Physiol.* **237**, 785.

The following two papers will appear in the *Symposium of Quantitative Biology*, of the Long Island Biological Association, vol. 4, 1936.

GRUNDFEST, H. "Excitation and recovery in nerve as modified by high pressure."

BROWN, D. E. S. "The sequence of events in the contraction of muscle at high pressure."

ÜBER DIE TIERGEOGRAPHISCHEN VERHÄLTNISSE DER CIRCUMANTARKTISCHEN SÜSSWASSERFAUNA

VON DR V. BREHM

(Received January 10, 1936)

Bei den Vorarbeiten zu einer Tiergeographie des Süßwassers drängte sich dem Verfasser oft der Versuch auf, die Verbreitungsbilder mit der Wegenerschen Verschiebungstheorie in Einklang zu bringen. Gelegentlich machte ich bei der Beschreibung neuer Arten oder neuer Fundstellen auf derartige Versuche aufmerksam, ebenso bei Vorträgen an der Biologischen Station Lunz. Im folgenden möchte ich nun wenigstens für das circumantarktische Gebiet eine kleine Zusammenstellung von Verbreitungsdaten mitteilen, die eventuell im Sinne der Wegenerschen Theorie betrachtet werden können. Bisher wurde von zoologischer Seite¹ solchen Betrachtungen noch wenig Beachtung geschenkt. Michaelsen (1922) hat die Oligochaeten zum Prüfstein der genannten Theorie gemacht und in diesen ein Beweismittel für dieselbe gesehen. Sickenberg (1934) hat die tertiären Landsäugetiere unter dem Gesichtspunkt der Verschiebungslehre durchmustert und kam zu dem Resultat, dass das von ihm studierte fossile Material zwar die Annahme der Wegenerschen Theorie gestatte, aber keinen bündigen Beweis für dieselbe bilde. Für die Süßwassertierwelt liegt meines Wissens noch keine solche Zusammenstellung vor, weshalb hier eine solche versucht werden soll; sie wird kein neues Tatsachenmaterial bieten, aber bei der riesig zerstreuten Literatur vielleicht für tiergeographisch orientierte Hydrobiologen nicht unwillkommen sein.

Von botanischer Seite hat man sich intensiver mit der Anwendung der Wegenerschen Theorie auf pflanzengeographische Probleme befasst und insbesondere mag auf Irmschers (1922, 1929) zweibändige Darstellung verwiesen sein, in der die Blütenpflanzen und die Laubmoose als Beweismittel zu Gunsten der Theorie Wegeners Verwendung finden. In der Gruppierung des Stoffes schliesse ich mich der Darstellung Irmschers an und überlasse es dem Leser in den mitgeteilten Daten weitere Stützen der Irmscherschen Gedankengänge zu sehen oder das Material mit einem "non liquet" einfach zur Kenntnis zu nehmen.

Bei dem Zurückgreifen auf die Darstellung Irmschers liegt mir der Gedanke nahe, ein Prinzip zur Anwendung zu bringen, das ich bereits in einer früheren Arbeit (Brehm, 1911) als Prinzip der Arealgleichheit eingeführt habe, womit folgendes gemeint sein soll. Wir sind bei der Betrachtung der tiergeographischen Verhältnisse der Süßwasserfauna in den meisten Fällen auf das Verbreitungsbild rezenter Formen angewiesen, da fossiles Material gewöhnlich fehlt. Über Ausnah-

¹ Es sei noch auf Meyrick Edward: "Wegeners hypothesis and the distribution of Micro-Lepidoptera", *Nature*, London, 1925, aufmerksam gemacht.

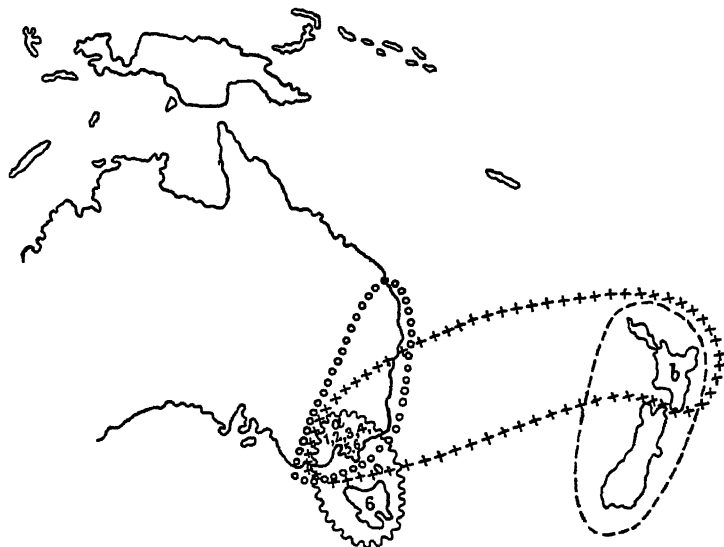
men hiervon siehe Seite 481, 489. Dadurch ist man gegenüber solchen Tiergruppen, für die fossile Belege ihrer früheren Verbreitung vorliegen, sehr im Nachteil. Doch lässt sich dieser Nachteil vielleicht beheben, wenn wir kongruente Verbreitungsbilder nachweisen können einerseits für eine solche Gruppe, die uns nur rezent vorliegt und andererseits für eine Gruppe deren heutiges Verbreitungsbild durch fossile Funde in seinem Zustandekommen verständlich gemacht werden kann. Wenn ich also im folgenden die Gleichheit der Areale irgendwelcher Süßwasserorganismen mit dem einen oder anderen Areal der von Irmscher studierten Pflanzen dartue, so geschieht dies in der Absicht, die von Irmscher gezogenen Schlüsse auch auf mein Material zu übertragen. Und das Aufsuchen solcher Arealgleichheiten scheint mir eine biogeographisch dankbare Aufgabe zu sein.

Das wichtigste Tatsachenmaterial für die Tiergeographie überhaupt und für die Verschiebungstheorie im Besonderen liegt uns wohl in den Disjunktionen vor, die demnach auch den Hauptteil der folgenden Ausführungen in Anspruch nehmen. Vorher möchte ich aber das Augenmerk auf eine Reihe australischer Endemismen lenken, die übrigens auch vielfach Disjunktionen, wenn auch mehr lokaler Natur, aufweisen. Denn gerade die Sonderstellung der australischen Organismenwelt hat sich für die Tiergeographie wie für die Verschiebungstheorie als sehr bedeutsam erwiesen. Und da sie vor allem für die höchststehenden Wirbeltiere sehr ausgeprägt und lange bekannt ist, spielt sie schon in älteren tiergeographischen Darstellungen (Wallace!) eine grosse Rolle. Weil man selbst heute noch vielfach auf die Ansicht stösst, dass die Süßwasserfauna ziemlich kosmopolitisch sei, sei gleich darauf hingewiesen, dass Fälle, wie die Verbreitung der Monotremen, auch in der niederen Tierwelt ihre Parallele haben.

Doch möchte ich da auf eine schon von Simroth diskutierte und vor kurzem auch von mir behandelte Sache aufmerksam machen (Brehm, 1932a). Man muss nämlich beachten, dass die tiergeographische Valenz der systematischen Kategorien keineswegs die gleiche ist. Während wir unter den Mammalien die Monotremen als australische Endemismen antreffen, sind es unter den Copepoden oder Cladoceren nur einzelne Gattungen. Deren tiergeographische Valenz wird aber sogleich klar, wenn man sich vor Augen hält, dass die meisten Gattungen dieser Kleinkrebse ausgesprochene Kosmopoliten sind.

Wenn wir ausgesprochen australische Typen Revue passieren lassen, so zeigt sich, dass wir neben solchen Formen, die über das ganze australische Gebiet verstreut sind, auch solche finden, die nur dem australischen Kontinent angehören, oder nur der einen oder anderen grossen Insel oder dass der Kontinent mit dieser oder jener Insel gemeinsame Formen aufweist. Über die Rolle, die in dieser Hinsicht Tasmanien, Neu Seeland und Neu Guinea spielen, unterrichtet jede grössere zoogeographische Darstellung und ich begnüge mich auf die beigegebenen Karten Nr. 1 und Nr. 2 zu verweisen.

Wir sehen da, dass als ausschliesslich dem australischen Kontinente eigen das Kopepodengenuss *Hemiboeckella* mit der einzigen Species *Searli* Sars und die zu den Macrotrichiden gehörige Cladocere *Neothrix armata* Gurney in Betracht kommen. Auf Neu Seeland beschränkt ist die Gattung *Metaboeckella*, die wieder nur durch



Karte 1. Verbreitung der Kopepodengenera *Calamoecia* Sars, *Brunella* Smith, *Hemiboeckella* Sars und *Metaboeckella* Sars nach dem gegenwärtigen Stand unserer Kenntnisse.

----- *Metaboeckella dilatata* Sars.

oooooooo *Hemiboeckella Searli* Sars.

++++ *Calamoecia*: a = *australica* Sars, b = *Lucasi*.

~~~~~ *Brunella*: 1 = *viridis* Searle, 2 = *longicornis* Searle, 3 = *australis* Searle, 4 = *ampulla* Searle, 5 = *expansa* Sars und 6 = *tasmanica* Smith.



Karte 2. Verbreitung der Cladocerengattungen *Saycia* Sars *Neothrix* Gurney und *Pseudomoina* King, sowie des Kopepodengenens *Gladioferens* Henry.

~~~~~ *Saycia orbicularis* Sars und *Pseudomoina lemnae* King.

oooooooo *Neothrix armata* Gurney.

----- *Gladioferens*: 1 = *spinus* Henry, 2 = *brevicornis* Henry, 3 = *gracilis* Kief.

eine Species, nämlich *dilatata* Sars, vertreten ist. Während also die nicht disjunkten Formen uns als monotype Gattungen entgegentreten, sehen wir, dass die disjunkten Formen eine mehr oder weniger weitgehende Artenzersplitterung zeigen. So ist von den Australien und Neu Seeland gemeinsamen Gattungen die kleine zarte Kopepodengattung *Calamoecia* auf dem Kontinent durch die Art *australica* Sars vertreten, auf Neu Seeland durch *Lucasi*; eine zweite Kopepodengattung, *Gladioferens*, besitzt in Ostaustralien die beiden Arten *spinosus* Henry und *brevicornis* Henry, während auf Neu Seeland die Art *gracilis* Kiefer lebt. Nur für die Cladoceren gilt dies nicht, da die beiden Gattungen *Saycia orbicularis* Sars und *Pseudomoina lemnae* King, die ich beide für Neu Seeland nachweisen konnte, monotyp sind. Hingegen zeigt sich die Zersplitterung sogleich wieder bei der Kopepodengattung *Brunella*, von der uns die Karte 6 Arten aufweist, die alle im südlichen Australien vorkommen und eine von diesen auch auf Tasmanien. Ich möchte an dieser Stelle bei der Kennzeichnung des endemischen Charakters der Süßwasserfauna der australischen Region nicht verabsäumen, auf einen Irrtum aufmerksam zu machen, der sich in meiner Mitteilung über diese Fauna (Brehm, 1928) befindet. Ich habe dort unter dem Namen *Antipodiella Chappuisi* eine Harpacticidengattung als Inselendemismus von Neu Seeland beschrieben, die von K. Lang als identisch mit *Epactophanes Richardi* erkannt wurde. Doch fehlt es nicht an endemischen Species, die ich aber hier bei Seite lasse, um mich auf die Gattungsendemismen zu beschränken, obwohl nach den oben über die tiergeographische Valenz der systematischen Kategorien gemachten Bemerkungen auch endemischen *Arten* keine geringe Bedeutung zukommt, etwa dem ganz isolierten *Simocephalus obtusatus* Thoms von Neu Seeland. Bevor wir uns den Grossdisjunktionen zuwenden, muss ich noch auf einen Übelstand aufmerksam machen, den auch unsere Karte zeigt, nämlich, dass wir von der Süßwasserfauna Neu Guineas fast gar nichts wissen. Wenn man bedenkt, dass hier nicht nur sehr wichtige Endemismen aus dem Reich der Wirbeltiere heimisch sind oder Formen, die wichtige Beziehungen zur australischen Fauna zeigen—Monotremata!—so muss man sagen, dass uns eine sichere Beurteilung der oben erwähnten australischen Entomotraken erst möglich sein wird, bis wir über deren Vorkommen oder Fehlen auf Neu Guinea Kenntnis haben. Es wird z. B. tiergeographisch wichtig sein, zu erfahren, ob Neu Guinea der Boeckellidenzone oder Diaptomidenzonen angehört oder ob dort beide sonst so scharf getrennten Gruppen eine Mischfauna bilden. Mit Rücksicht auf das Verhalten der Marsupialier hätte man auch bei Celebes mit der Möglichkeit des Vorkommens irgendwelcher Boeckelliden rechnen können. Aber Wolterecks Durchforschung der Binnengewässer von Celebes zeigte, dass diese Insel noch ganz der Diaptomidenzonenregion angehört.

Unter den Grossdisjunktionen sind seit langem die auffallenden Übereinstimmungen zwischen der australischen und patagonischen Fauna Gegenstand wissenschaftlicher Erörterung gewesen. Man kann die vielen Erklärungsversuche, die hierfür gemacht wurden, etwa in drei Kategorien einteilen:

1. Solche Theorien, welche diese Übereinstimmung darauf zurückführen, dass sie annehmen, beide Faunengebiete seien die Überreste eines ursprünglich

zusammenhängenden, einheitlichen antarktischen Faunengebietes, wobei der Zusammenhang entweder durch eine Landbrücke (Arlt) oder durch direkten Kontakt (Wegener) gegeben ist.

2. Solche Theorien, welche die Übereinstimmung darauf zurückführen, dass beide Gebiete die gemeinsamen Formen aus einem einheitlichen Ursprungsgebiet auf der nördlichen Halbkugel bezogen hätten (Simroth).

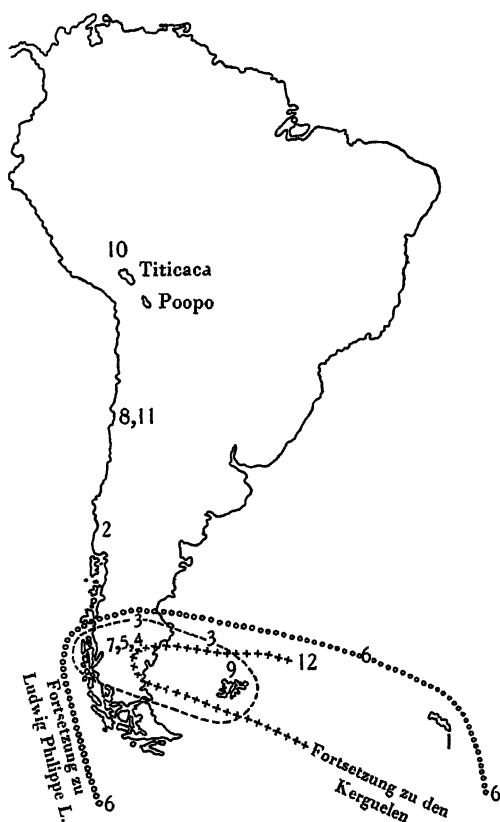
3. Solche, die die Übereinstimmungen lediglich als Konvergenzerscheinungen auffassen wollen.

Wir wollen gleich vorweg nehmen, dass der dritte Fall, wenn er überhaupt in Frage kommen sollte, nur in Ausnahmefällen zutreffen dürfte. Für die Zurückführung auf eine einheitliche Quelle—einerlei ob sie auf der nördlichen Halbkugel oder auf der Antarktica zu suchen wäre—haben schon frühere Autoren die interessante Erscheinung geltend gemacht, dass patagonische und australische Organismen mehrfach von identischen Parasiten bewohnt werden. Es sei an die Cestodengattung *Linstowia* erinnert, die australische und südamerikanische Marsupialier bewohnt, an den analogen Fall der Trematodengattung *Harmostomum* und an die Opalinidengattung *Zelleriella*, die nur in den in Südamerika und Australien heimischen Fröschen der Familie der Leptodactyliden vorkommt. Die Pflanzenwelt steuert ein analoges Beispiel in der Ascomycetengattung *Cyttaria* bei, die als Nothofagus-schmarotzer beiden so weit getrennten Wohngebieten angehört.

Hier ist wohl der Schluss auf einen gemeinsamen Herd der Herkunft unabweisbar.

Schalten wir also die Annahme aus, dass die Übereinstimmung zwischen der australischen und südamerikanischen Lebewelt nur der Ausdruck konvergenter Entwicklungsprozesse sei, so stehen wir bei der Annahme, dass die Übereinstimmungen auf einen ehemaligen Zusammenhang der heute disjunkten Wohngebiete zurückzuführen seien. Und damit stehen wir vor der Frage, ob dieses ursprüngliche Wohngebiet der nördlichen Halbkugel angehörte oder dem Bereich der Antarktica. Dass tatsächlich viele Organismen, deren Wohngebiete heute dem Südpol benachbart sind, ihren Ursprung auf der nördlichen Halbkugel hatten, ist durch fossile Funde belegt und bildet ja einen Hauptstützpunkt der von Haacke (1896) und Simroth (1907) entwickelten Theorien. Eine andere Frage ist es, ob man mit den genannten beiden Autoren diese Annahme auf die ganze Organismenwelt ausdehnen darf. Da die Ableitung von der nördlichen Halbkugel sich in erster Linie auf palaeontologisches Material stützen muss, der Grossteil der Süßwasserfauna aber zur Fossilbildung nicht geeignet ist, können wir wohl nur von Wirbeltieren und Mollusken des Süßwassers Belege erwarten. Solche liegen auch in der Tat vor. So ist die durch ihre Ancyclus-ähnlichen Gehäuse gekennzeichnete Gattung *Gundlachia* heute nur in Südamerika und Tasmanien vertreten, lebte aber im Untermiocen in Mitteleuropa: *Gundlachia francofurtana*. Und unter den Wirbeltieren bietet *Ceratodus* ein analoges Beispiel. Man hat aber bei diesen Betrachtungen nicht nur Fossilien der nördlichen Halbkugel als Belege verwendet, sondern auch "lebende Fossilien" der nördlichen Halbkugel. Hierfür bietet die Süßwasserfauna ein besonders auffallendes Beispiel in der *Boeckella orientalis* Sars.

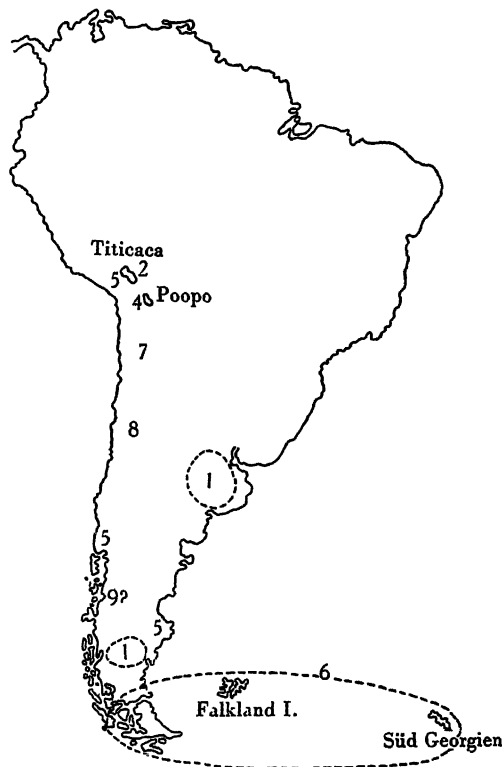
Wie unsere Karten Nr. 3 und 4 und Tabelle der Seite 485 zeigen, sind die Boeckelliden eine ganz auf die australische und südamerikanische Region beschränkte Kopepodenfamilie, so dass die Angabe vom Vorkommen eines Vertreters dieser Familie in der Mongolei lange Zeit angezweifelt und auf eine Materialverwechslung zurückgeführt wurde, bis sie sich als Tatsache bestätigte. Übrigens hat dieser Fund in neuester Zeit ja eine Parallele bei der Landfauna gefunden durch die ebenfalls in Innerasien glückte Entdeckung eines Peripatiden, des *Typhloperipatus Williamsoni*; wenn auch



Karte 3. Verbreitung der südamerikanischen Arten der Gattung *Pseudoboeckella* (vgl. die Tabelle II auf Seite 484).

die Peripatiden nicht circumantarktisch sind, wie die Boeckelliden, so repräsentieren sie doch eine Gruppe deren Verbreitung fast ganz der südlichen Halbkugel angehört, so dass von manchen Autoren das Peripatidenareal mit dem Glossopterisareal verglichen wurde. Vielleicht wird man auch das Verbreitungsbild der Syncariden hierherrechnen dürfen, eine Gruppe die wir heute fast ganz als australisch bezeichnen müssten (*Anaspides*, *Koonunga*), wenn nicht in Europa palaeozoische Vertreter dieser Gruppe und die Gattungen *Bathynella* und *Parabathynella* als lebende Fossilien für die Herkunft der tasmanischen Formen vom Norden her sprechen würden. Dass man bei derlei Betrachtungen eine gewisse Vorsicht walten lassen muss, zeigt

allerdings die Gattung *Daphniopsis*, die bisher nur aus Tibet und von den Kerguelen bekannt ist, so dass man versucht sein könnte in der tibetansichen Form eine Parallele zur oben genannten *Boeckella* zu sehen. Aber die Gattung *Daphniopsis* ist wohl nur eine künstliche Gattung und die Übereinstimmung der Kerguelenform *D. Studeri* und der tibetanischen *D. tibetana* ist wohl nichts anders als eine Konvergenzbildung, sowie ja auch die grosse Übereinstimmung des *Canis antarcticus* von den Falkland-Inseln mit dem nordamerikanischen *C. latrans* von den meisten



Karte 4. Verbreitungskarte der südamerikanischen Arten der Gattung *Boeckella* (vgl. die Tabelle I auf Seite 485).

Autoren als Konvergenzbildung aufgefasst wird und nichts über die Herkunft der Falklandart sagt.

Wenn nun—und das trifft gerade auf die Süsswasserfauna in hohem Grade zu—in vielen Fällen keine fossilen Belege für die Annahme einer Herkunft von der nördlichen Halbkugel vorliegen, so wird von Autoren wie Simroth oder Haacke diesem Beweismangel keine Bedeutung beigemessen, da man darauf verweist, dass die Auffindung von fossilen Belegen denn doch grossenteils Glückssache sei und für viele Organismen gar nicht gefordert werden dürfe, weil sie nicht für fossile Erhaltung geeignet sind. Dem gegenüber möchte ich einen Fall aus der Süsswasserfauna erwähnen, der mir zu beweisen scheint, dass doch in manchen Fällen Ende-

mismen der südlichen Halbkugel auf dieser autochthon seien. Die Süßwassermuschel *Diplodon* lebt heute in Südamerika, Australien, Neu Seeland und Tasmanien. Trotz des überreichen fossilen Materiales von Süßwassermuscheln, das wir auf der nördlichen Halbkugel und speziell in Nordamerika finden, wurde hier noch nie ein *Diplodon* gefunden, was gegen die restlose Herleitung der circumantarktischen Organismen von der nördlichen Halbkugel spricht. In der gleichen Weise wird wohl auch die Existenz von Gattungen zu deuten sein, die selbst heute—also unter denkbar ungünstigen Bedingungen—auf das Randgebiet der Antarktica und die diesem vorgelagerten Inseln beschränkt sind. Ein bekanntes Beispiel dieser Art ist die monotype Moosgattung *Sarconeuron* von Grahamland und Victorialand, der sich vielleicht die Collembolengattung *Tullbergia* anreihen liesse, die vom Kerguelen-Crozet-gebiet, Süd-Georgien und aus der Westantarktis bekannt ist. Auch die Süßwasserfauna dürfte einen entsprechenden Fall aufweisen, nämlich die Harpacticidengattung *Antarctobiotus* Chapp. Im Jahre 1908 beschrieb Richters im ix. Band der Deutschen Südpolarexpedition einen *Canthocamptus robustus*, der in Moosrasen auf der Insel Possession bei Victoria erbeutet wurde und 1928 beschrieb O. Pesta (1928) von Süd-Georgien eine *Atteyella Koenigi*. Wie nun Chappuis (1930) zeigte, sind diese beiden Formen Vertreter einer neuen Gattung, die er *Antarctobiotus* nannte und die in ihrer Verbreitung streng antarktisch zu sein scheint. (Vgl. Karte Nr. 8.) Bei den überaus ungünstigen Lebensbedingungen, welche heute im antarktischen Gebiete herrschen, ist wohl nur mit vereinzelt Fällen solcher extrem antarktischer Typen zu rechnen. Aber für die Beurteilung der Frage nach der Herkunft der Organismenwelt sind auch diese wenigen Fälle von grosser Bedeutung und sie sprechen wohl auch gegen die Meinung Dahls, dass die Antarktis ein durchaus künstliches Gefüge hinsichtlich seiner Fauna darstelle und nur Ausläufer der benachbarten Kontinente beherberge. (Vgl. Dahl, F., *Grundlagen einer ökologischen Tiergeographie*, Bd. 2, Seite 106.)

Betrachten wir nun unter Ausserachtlassung der Frage nach der jeweiligen Herkunft die disjunkten Areale verschiedener Tiergruppen der circumantarktischen Fauna, so fallen zuerst jene zahlreichen Fälle auf, in denen die australische Region mit der südamerikanischen verknüpft erscheint.

Tabelle I. *Pseudoboeckella*arten, zugleich Legende für die Karte Nr. 3

1. *Poppei* Mraz. Südliches Patagonien und Süd-Georgien
2. *Klutei* Brehm. El Junco. 40°
3. *brasiliensis* Lubbock. Patagonien und Feuerland 48° bis 55°
4. *dubia* Dad. Ostpatagonien bei 50°
5. *Silvestrii* Dad. Patagonien, 50°
6. *Entzi* Dad. Patagonien, Feuerland, Falkland, Süd-Georgien, Ludwig-Philipp-Land
7. *longicauda* Dad. Patagonien
8. *erubescens* Brehm. Chile bei 33°
9. *Valentini* Scott. Falkland-Inseln
10. *Godeti* Delachaux. Peru.
11. *gibbosa* Brehm. Santiago in Chile
12. *brevicauda* Mraz. Patagonien, Falkland-Inseln, Kerguelen

Anmerkung. Bezüglich der Arten 9, 10, 11 bestehen Zweifel, ob sie der Gattung *Pseudoboeckella* zugerechnet werden dürfen. Durch diese Arten wird die Selbständigkeit dieser Gattung in Frage gestellt. Immerhin bleibt von Interesse, dass keine eigentliche *Pseudoboeckella* im australischen Gebiet vorkommt. Ferner wäre zu beachten, dass *Klutei* mit *Poppei* nächstverwandt ist und *erubescens* mit *Silvestrii*, endlich *gibbosa* mit *Valentini*.

Tabelle II. *Boeckellaarten, zugleich Legende für die Karte Nr. 4*

| Südamerika | Australien |
|---|---|
| 1. <i>Bergi</i> Rich. Vom La Plata südwärts bis 40°; ein zweites Wohngebiet bei etwa 50° | 1. <i>triarticulata</i> Thoms. S.O. Australien, Neu Seeland |
| 2. <i>occidentalis</i> Marsh. Titicaca, Poopo, Laguna de Junin | 2. <i>oblonga</i> Sars. Victoria. Südaustralien |
| 3. <i>gracilis</i> Dad. Nordostpatagonien | 3. <i>Saycei</i> Sars. Victoria. Südaustralien |
| 4. <i>poopensis</i> Marsh. Pooposee | 4. <i>symmetrica</i> Sars. Victoria. Südaustralien |
| 5. <i>gracilipes</i> Dad. Titicaca, Chile bei 40°, Patagonien bei 50° | 5. <i>robusta</i> Sars. N.S. Wales |
| 6. <i>Michaelsemi</i> Mraz. = <i>pygmaea</i> Dad. = <i>Anderssonorum</i> Ekm. Patagonien, Feuerland, Falkland, Süd-Georgien | 6. <i>minuta</i> Sars. Victoria, N.S. Wales |
| 7. <i>Rahmi</i> Brehm. Chile bei 22° | *7. <i>propinqua</i> Sars. Neu Seeland |
| 8. <i>dentifera</i> Brehm. Chile bei 33° | 8. <i>asymmetrica</i> Searle. Melbourne |
| 9. <i>meteoris</i> Kiefer. Glencross in Patagonien | 9. <i>coronaria</i> Henry. N.S. Wales |
| | 10. <i>fluvialis</i> Henry. N.S. Wales |
| | 11. <i>insignis</i> Smith. Tasmanien |
| | 12. <i>longiseta</i> Smith. Tasmanien |
| | 13. <i>nyoraensis</i> Searle. Victoria |
| | 14. <i>pseudocheles</i> Searle. Victoria |
| | 15. <i>rubra</i> Smith. Tasmanien |
| | 16. <i>tenera</i> Sars. Victoria und Südaustralien |
| | 17. <i>hamata</i> Brehm. Lake Lyndon, Neu Seeland |

* *Propinqua* ist möglicherweise identisch mit *triarticulata*.

Die Koniferengattungen *Araucaria*—diese wieder mit fossilen Belegen ihrer ursprünglichen Heimat auf der nördlichen Halbkugel, sowie *Fitzroya* sind oft zitierte Beispiele, ebenso die ausgestorbenen gehörnten Schildkröten der Gattung *Miolania*. Diesen Fällen reiht sich innerhalb der Süßwasserfauna vor allem die Familie der Boeckelliden an. Seit der letzten zusammenfassenden Darstellung dieser Familie durch Marsh (1924) sind so viele neue Funde hinzugekommen, dass es sich lohnt, eine tabellarische Übersicht über dieselben zu geben. Unsere Tabellen I und II zeigen, dass zwar auf der amerikanischen Seite sich besondere Untergattungen heraus differenziert haben—*Pseudoboeckella* und *Parabroteas*¹—dass aber neben diesen zahlreiche Vertreter der in Australien weitverbreiteten typischen *Boeckella* auch in Amerika vorkommen. Noch mehr verliert dieser Unterschied an Gewicht, wenn man bedenkt, dass die Trennung der Gattungen *Boeckella* und *Pseudoboeckella* recht problematisch ist. Vgl. darüber Delachaux (1928) und Brehm (1935b) und die Anmerkung auf Seite 484.

Auf der australischen Seite kennen wir bisher Boeckelliden nur aus dem südöstlichen Teil des Kontinentes, von Tasmanien und Neu Seeland, also von den der Antarktis zugewendeten Teilen. In Südamerika liegt der Schwerpunkt der Verbreitung auch in dem der Antarktis zugekehrten Teil, nämlich in Patagonien und den benachbarten Inseln, ja eine Art ist von der Antarktis selbst bekannt, nämlich *Pseudoboeckella Entzi* Daday.

Von diesem Hauptgebiet aus haben hier die Boeckelliden (vgl. Karten 3 und 4), die tief temperierten Hochgebirgsseen der Anden benützend, bis ins Gebiet des Titicaca vorstossen können, was mit Rücksicht auf ihr ökologisches Verhalten nicht verwunderlich ist. Zwei Arten haben aber im Bereich des kühleren Klimas auch in das argentinische Tiefland sich verbreiten können, wo der Rio de la Plata dem Vorstoss nach Norden ein Ziel setzt. Wenn wir schon den Verbreitungsbildern der südamerikanischen Boeckelliden unser Augenmerk schenken, so möchte ich noch

¹ Dessen Verbreitung ist aus der Anmerkung auf Seite 487 zu ersehen.

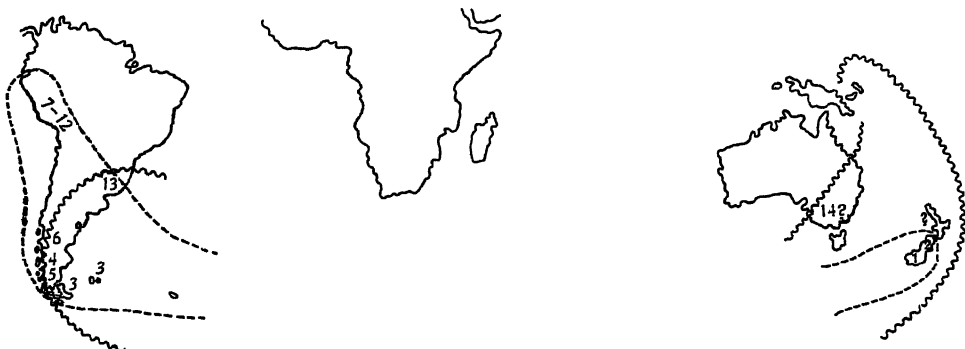
auf das sehr ungleiche Verhalten der einzelnen Arten hinsichtlich der Grösse des von ihnen bewohnten Areales aufmerksam machen. An erster Stelle steht da wohl *Pseudoboeckella brevicaudata*, die von Patagonien über das Gebiet der Falkland-Inseln zu den Kerguelen reicht, das einzige Beispiel unter den Boeckelliden, dass eine Art der östlichen und westlichen Halbkugel angehört. Ihr folgt *Pseudoboeckella Entzi*, die von Patagonien bis Süd-Georgien vorkommt und zugleich als einzige bisher aus der Antarktis selbst bekannte Art in Ludwig-Philipps-Land angetroffen wurde. Ihr in der Verbreitung gleich ist *Boeckella Michaelsoni*, die aber auf dem antarktischen Kontinent selbst bisher nicht gefunden wurde. Sehr gross, wenn auch nur vom südamerikanischen Kontinent selbst bekannt ist das Areal von *Boeckella gracilipes*, das eine Spannweite von 30 Breitengraden aufweist, da diese Art vom Titicaca und aus Patagonien bekannt ist. Etwas kleiner ist die Spannweite zwischen den beiden Arealen der *Boeckella Bergi*, die wohl die Aussenflügel eines einzigen zusammenhängenden Wohngebietes darstellen dürften. Diese weiten Areale überraschen deshalb, weil die grosse Menge der übrigen Boeckelliden sehr eng begrenzte Wohngebiete zu haben scheint. Die Neigung zur Bildung von Endemismen innerhalb dieser Familie mag nicht überraschen, wenn man die Variabilität einzelner Populationen in Betracht zieht. Man vergleiche hierüber Brehm (1935 b). In vielen tiergeographischen Werken wird betont, dass die Ähnlichkeit mit der australischen Fauna nur für die Fauna des südlichsten Südamerika gelte; aber wie in vielen anderen Fällen zeigt auch hier unsere Karte ein weites Ausgreifen nach Norden, das jedesfalls ökologisch bedingt ist. Die Hochgebirgsseen der Anden gestatten der patagonischen Fauna dieses Vordringen. Dass zwei Arten *Boeckella Bergi* und *B. gracilis* auch das argentinische Flachland besiedeln konnten, scheint auf eine ökologische Sonderstellung dieser Arten schliessen zu lassen. Doch haben diese nordwärts das Mündungsgebiet des Rio de la Plata nicht überschritten, bleiben also weit hinter dem Boeckellengebiet des gebirgigen Westens zurück. Vergl. Brehm (1935 b).¹

Während die Boeckelliden auf der australischen Seite über den australischen Kontinent, Tasmanien und Neu Seeland verbreitet sind—vgl. Tabelle II—scheinen andere Gattungen dort nicht so allgemein verbreitet zu sein. Irmscher macht darauf aufmerksam, dass hier verschiedene Disjunktionen auf botanischem Gebiet realisiert sind; so ist die auffallende Moosgattung *Dendrooligotrichum* nur von Südamerika und Neu Seeland, andere wieder nur von Südamerika und Tasmanien bekannt, u. s. w. Wenn im folgenden zwei Fälle dieser Art aus der circumantarktischen Süsswasserfauna angeführt werden, so muss doch noch mit der Möglichkeit gerechnet werden, dass diese Bilder nur ein Ausdruck unserer unvollkommenen Kenntnis der australischen Süsswasserfauna sind und dass bei weiterer Durchforschung sich diese Gattungen in Australien als weiter verbreitet

¹ Wenn die Annahme einer nördlichen Herkunft der Boeckelliden korrekt ist—und sie dürfte es sein—so dürfte man eigentlich in den Arten des Titicacagebietes oder des Gebietes südlich der La Plata-Mündung nicht nach Norden vorstossende Arten erblicken, sondern auf der Südwanderung zurückgebliebene Formen. Diese Annahme könnte durch gewisse morphologische Übereinstimmungen mit der Art *orientalis* gestützt werden. Schon Marsh betonte, dass *occidentalis* der mongolischen Art *orientalis* weit ähnlicher ist als den übrigen amerikanischen Arten.

erweisen könnten, so dass ihre Areale etwa mit dem der Boeckelliden verglichen werden könnten.

Der eine Fall betrifft die Harpacticidengattung *Delachauxiella* Chapp. Wie unsere Karte 5 zeigt ist diese Gattung in Südamerika in der gleichen Weise verbreitet wie die Boeckelliden, aber in der australischen Region mit Sicherheit nur von Neu Seeland bekannt. Dort leben die beiden Arten *D. Bennettii* Brehm und *Brehmi* Chapp., zu denen sich vielleicht noch zwei weitere gesellen, die ich als *maoricus* und *misogynus* beschrieben habe, deren Zugehörigkeit zur Gattung *Delachauxiella* dadurch unsicher ist, dass beide nur im männlichen Geschlecht aufgefunden wurden. Die Zweifel an der Beschränkung dieser Gattung auf Neu Seeland sind gegeben durch eine Angabe Chappuis, der vermutet, dass die von



Karte 5¹. Verbreitung der Harpacticidengattung *Delachauxiella* Brehm und der Ostrakodengattung *Newnhamia* King.

----- *Delachauxiella*. ~ ~ ~ ~ *Newnhamia*. 1 = *Bennettii* Brehm, 2 = *Brehmi* Chapp., 3 = *trigonura* Ekm., 4 = *Dadayi* Chapp., 5 = *Horwathi* Chapp., 6 = *Hamae* Kiefer, 7 = *aculeata* Thieb., 8 = *insignis* Del., 9 = *maxima* Del., 10 = *ensifer* Del., 11 = *lanceolata* Del., 12 = *ferox* Del., 13 = *lanata* Mraz., 14 = ? (= *Moraria longiseta*).

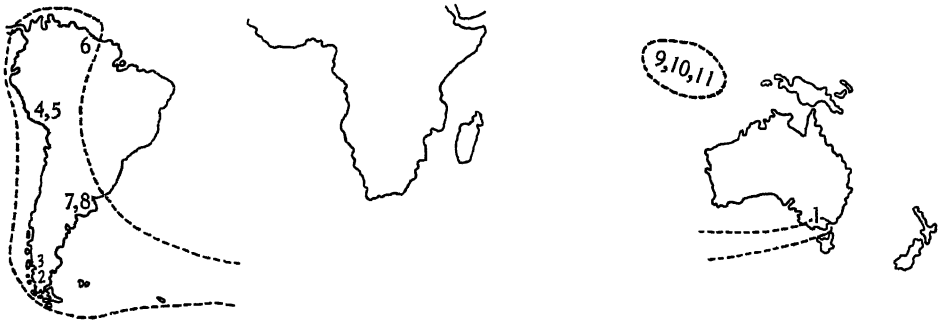
Henry beschriebene *Moraria longiseta* aus Südostaustralien, die bestimmt keine *Moraria* ist, zur Gattung *Delachauxiella* gehören möge.

Umgekehrt ist die Gattung *Chappuisiella* Brehm vom australischen Kontinent, aber nicht von Neu Seeland bekannt. Ferner unterscheidet sich der Fall *Chappuisiella* vom vorigen dadurch, dass von den Sunda-Inseln einige Arten bekannt sind, die zwar von den südamerikanischen und australischen Typen abweichen, aber

¹ Erst nach der Fertigstellung dieser Karte fand ich im Material aus der Laguna del Inca (2800 m.), der Laguna cristallina (3400 m.) und aus einem unbenannten, 3300 m. hoch gelegenen Gewässer, das ebenfalls, wie die zwei zuerst genannten Seen, in der Cordillere bei Santiago liegt, eine *Delachauxiella*, die ich in der inzwischen, 1936, erschienenen Mitteilung Nr. VII über die Forschungsreisen Prof. Rahms (*Zool. Anz.* 114, Seite 159) als *trigonura* namhaft machte. Indessen überzeugte ich mich, dass diese Kolonien zwar mit einer von mir ebenfalls als *trigonura* bezeichneten Form aus dem Gewässer El Junco (Brehm, 1925) identisch sind, nicht aber mit der typischen *trigonura* Ekman. In einer demnächst im *Zool. Anz.* erscheinenden Mitteilung glaube ich zeigen zu können, dass die echte *Delachauxiella trigonura* auf den äussersten Süden Südamerikas beschränkt ist, während die von mir bisher als *trigonura* gemeldeten Arten eine von *trigonura* verschiedene, auf die Hochseen der mittleren Anden beschränkte Form bilden dürften. Hingegen stellt der Nachweis von *Parabroteas Michaelsoni* Mrazek in meiner oben erwähnten Mitteilung VII eine unerwartete Erweiterung des bisher bekannten Wohngebietes dieser Art—Süd-Patagonien, Falkland-Inseln, Süd-Georgien—dar.

doch noch zu *Chappuisiella* zu rechnen sind. Ob wir in diesen Formen nach Norden vorstossende Arten einer Gattung der südlichen Halbkugel sehen, wie Chappuis will, oder Nachzügler der von der nördlichen Halbkugel stammenden *Chappuisiella*, bleibt eine offene Frage. Dem Vorkommen auf den Sunda-Inseln entspricht auf amerikanischer Seite ein Vorstoss bis an die Nordküste Südamerikas, wie unsere Karte 6 zeigt.

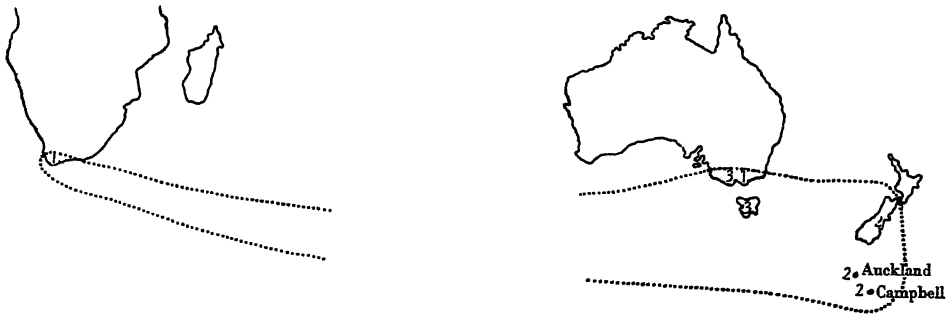
Ein ähnliches Verbreitungsbild, das zugleich an das Verhalten der Gattungen *Fagus* und *Nothofagus* erinnert, finden wir bei der Ostracodenfamilie der Noto-dromadiden. Bekanntlich ist *Nothofagus* rezent und fossil auf die südliche Halbkugel beschränkt, und zwar unter Auslassung Afrikas, während *Fagus* heute ein Bewohner der nördlichen Halbkugel ist. Auch die Notodromadiden bestehen aus zwei nahe verwandten Gattungen von gleichem Verhalten. Die Gattung *Noto-dromas* dürfte, soviel ich aus der Literatur ersehe, eine Form der nördlichen Halb-



Karte 6. Verbreitung der Harpacticidengattung *Chappuisiella* Brehm. 1 = *australica* Sars, 2 = *crenulata* Mraz., 3 = *occulta* Kiefer, 4 = *Godeti* Delach, 5 = *Huaronensis* Delach, 6 = *guianensis* Delach, 7 = *derelecta* Brian, 8 = *subdola* Brian, 9 = *Ruttneri* Chapp., 10 = *minuta* Chapp., 11 = *inopinata* Chapp.

kugel sein und dürfte mit der Art *N. Entzi* auf Ceylon ihre Südgrenze erreichen. Die zweite Gattung *Newnhamia* hingegen (vgl. Karte 5) gehört ganz der südlichen Halbkugel mit Ausschluss Afrikas an. Die Art *N. fenestrata* King erstreckt ihr Wohngebiet von Ostaustralien und Neu Seeland bis zum Bismarck-Archipel, die ungenügend beschriebene Art *fusca* gehört Südaustralien an, *Newnhamia patagonica* Vavra bewohnt das südlichste Südamerika und *N. Thomseni* ist aus Uruguay und vermutlich auch aus dem nahen Argentinien bekannt. Weniger bedeutsam scheint mir bei der weiten Verbreitung der Ostrakodengattung *Herpetocypris*, dass zwei einander offenbar recht nahestehende Arten, nämlich *Herpetocypris Pascheri* Brehm und *H. pectinata* Brehm einerseits von Neu Seeland, andererseits von Chile bekannt sind. Vgl. Brehm (1934). Während Fälle von Disjunktionen zwischen Südamerika und Australien für die Süßwasserfauna sicher noch durch weitere Beispiele zu belegen wären, scheinen solche zwischen Afrika und Australien sehr selten vorzukommen, was, wie die Ausführungen Irmschers zeigen, auch für andere Organismengruppen Geltung hat. Die Beschränkung auf Australien und Südafrika, die auf botanischem Gebiet durch den *Goniomitrium*typus unter den Moosen

repräsentiert wird, finden wir in der Süßwasserfauna bei der Amphipodengattung *Chiltonia* wieder (Karte 7); im australischen Gebiet finden wir drei Arten dieser Gattung, nämlich *australis* Smith in Victoria und auf Tasmanien, *mihiwaka* Chilton in Südastralien, auf Neu Seeland und auf den Auckland und Campbell-Inseln, die der Südinsel von Neu Seeland vorgelagert sind, und endlich *subtenuis* Sayce, die in Victoria entdeckt und später von Rühe (1901) in Südafrika wiedergefunden wurde. In Amerika ist die Gattung *Chiltonia* durch die nahe verwandte Gattung *Hyaella* vertreten, die aber nicht etwa auf den patagonischen Teil Amerikas beschränkt ist, sondern sogar in Nordamerika vorkommt und zwar in einer Art—*Hyaella azteca* = *H. Knickerbockeri*—die in identischen oder nahe verwandten Kolonien über ganz Amerika verbreitet zu sein scheint, während in Südamerika und speziell in den Andengewässern noch eine grosse Anzahl endemischer Arten vorzukommen scheint. Ob die nordamerikanischen Hyaellakolonien vielleicht Zeugen der Herkunft dieser Formen von der nördlichen Halbkugel sind, ist natürlich fraglich.

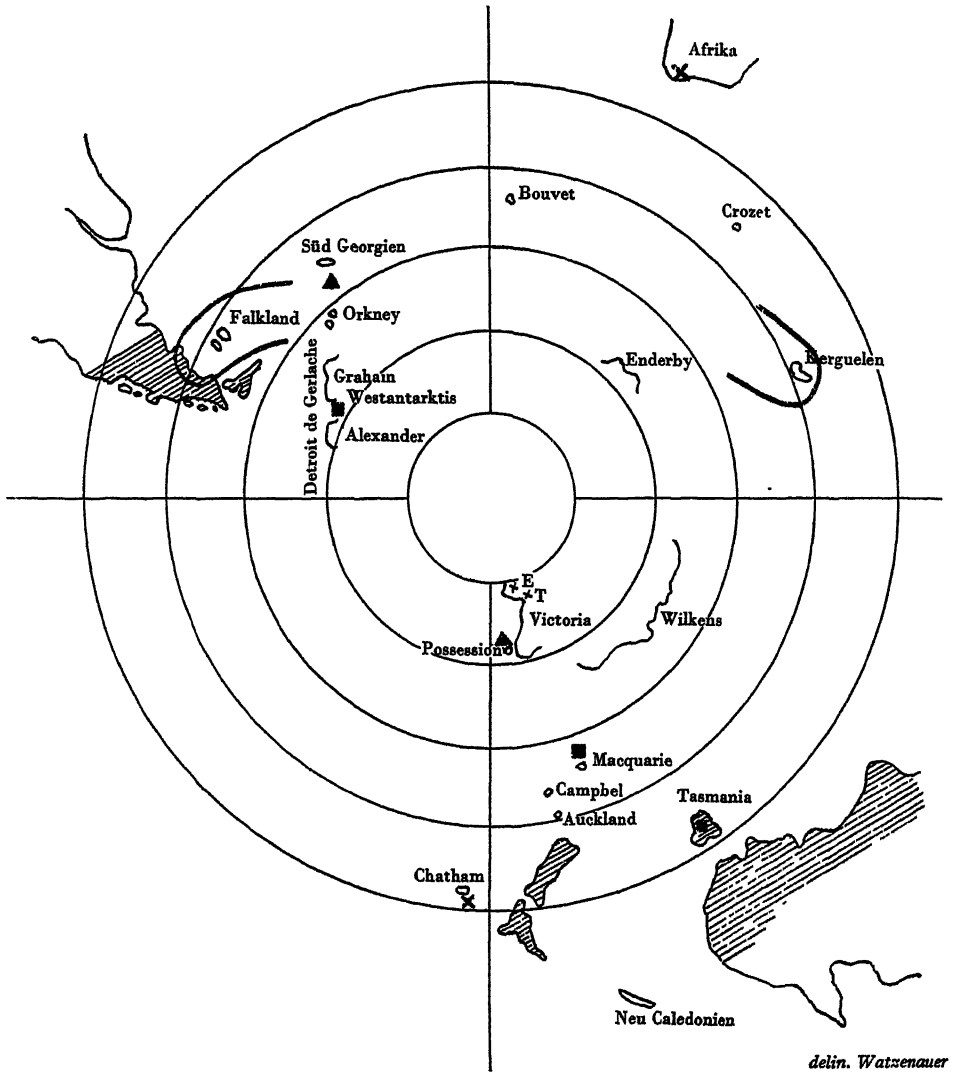


Karte 7. Verbreitung der Amphipodengattung *Chiltonia*. 1 = *subtenuis* Sayce, 2 = *mihiwaka* Chilton, 3 = *australis* Smith.

Einen eigenartigen Fall, für den ich bei Irmischer keine Parallele finde, repräsentiert die kürzlich von Schellenberg (1930) entdeckte Amphipodengattung *Falklandella* von den Falkland-Inseln. Bisher fehlte nämlich jeder sichere Anhaltspunkt für das Vorkommen von Gammariden in Südamerika, wo die Amphipoden im Süßwasser ausschliesslich durch die Talitriden vertreten zu sein scheinen. Das war deshalb sehr auffallend, weil die Gammariden sowohl in der australischen Region wie in Südafrika vertreten sind. Nun wurde—zwar nicht auf dem amerikanischen Kontinent wohl aber auf den Falkland-Inseln—von Schellenberg die oben genannte Gammaridengattung in zwei Arten *Falklandella obtusa* und *F. cuspidata* gefunden. Dabei zeigte sich, dass diese neue Gattung ein sehr bemerkenswertes Merkmal mit den übrigen Gammariden der südlichen Halbkugel gemein hat, nämlich den Besitz der Sternalkiemer, die bei Formen der nördlichen Halbkugel nur ausnahmsweise vorkommen und hier vielleicht auf einen engeren genetischen Zusammenhang hindeuten.

Während so für die Gammariden sich das Wohngebiet von der australischen und südafrikanischen Region auf Südamerika ausgedehnt erwies, scheinen gerade

gewisse für die südliche Halbkugel typische Isopoden Südamerika nicht erreicht zu haben. So hat *Phreatoicus*—in Südafrika vertreten durch die Art *capensis*—nur in Australien und Tasmanien nähere Verwandte, wobei die am weitesten von einander



Karte 8. Das circumantarktische Gebiet.

— *Pseudoboeckella brevicaudata*. ||||| *Galaxiidae*. ▲ *Antarctobiotus* Chapp.
 × *Laophonte chathamensis* Sars. ■ *Tigriopus angulatus* Lang.

wohnenden Arten, nämlich *capensis* und *australis* einander am nächsten stehen und zugleich nahe Beziehungen zu der ausgestorbenen Art *wianamattensis* zeigen. Die winzige, blinde *Janirella* Sayce, die 1900 in Victoria entdeckt wurde, hat jüngst einen Familiengenossen aus Südafrika erhalten, die *Protojanira Prenticei* Barnard,

die von ihrem Entdecker daselbst in Sphagnumrasen gefunden wurde. Aus Südamerika ist meines Wissens noch keine verwandte Form bekannt, was freilich nicht ausschliesst, dass eine solche noch gefunden werden könnte.

Absichtlich beschränken sich diese Mitteilungen auf die Süßwasserformen. Denn deren Disjunktionen verlangen einen ehemaligen Zusammenhang der heute getrennten Wohngebiete, während Bewohner von Binnengewässern, die mariner Herkunft sind, sehr gut die heute getrennten Wohnbezirke von einem zwischen diesen liegenden Meer aus erreicht haben können. Aus diesem Grunde möchte ich den in der Literatur oft zitierten—vgl. Simroth (1907), Arldt (1917–1922)—Beispielen der Galaxiiden (vgl. Karte 8) und Haplochitoniden oder der Cyclostomen-gattung *Geotria* keine zu grosse Bedeutung beimessen. Doch könnten auch in solchen Fällen, wenn es sich um weit auseinander liegende disjunkte Gebiete ein und derselben Species handelt, Schlüsse auf eine ehemalige nähere Verbindung der heute weit getrennten Wohngebiete zulässig sein, weil im gegenteiligen Falle doch statt der identischen Arten vikarierende zu erwarten wären. Ein solcher Fall liegt vielleicht bei dem Harpacticoiden *Laophonte chathamensis* Sars vor, der von seinem Entdecker in der Lagune Warekauri auf den Chatham-Inseln bei Neu Seeland gefunden und nachher von Rühe in Südafrika bei Zand Vlei nachgewiesen werden konnte. Gleichen Gedankengängen mag sich das Verbreitungsbild eines zweiten Harpacticoiden fügen, des *Tigriopus angulatus* Lang, den ich in dem Material von den Forschungsreisen Prof. Rahms gefunden habe, nachdem er von Lang (1934) von der Mündung des Brown River auf Tasmanien, von der Macquarie Insel und von Detroit de Gerlache festgestellt worden war (vgl. Karte 8). Unser Kartenbild illustriert die Worte die ich (Brehm, 1935 b) dem Bericht über diesen Fund anschloss: "Man sieht, dass das neue Vorkommen die direkte Fortsetzung einer Linie bildet, welche die bisher bekannten Fundstellen verbindet und welche die Verbreitungslinie darstellt, die den Faunenaustausch zwischen der australischen Region über die Westantarktis nach Südamerika vermittelt haben muss." Für beide Fälle vgl. Karte 8.

Sind schon die Beziehungen zwischen Australien und Afrika nur durch vereinzelte Beispiele zu belegen, so werden diese noch seltener, wenn man solche zwischen Südamerika und Afrika sucht. Auch Irmscher (1929) erwähnt auf Seite 275 des zweiten Bandes seines Werkes: "Für diese Arealform ist mir von höheren Pflanzen kein Beispiel bekannt"! Doch kann er für Bryophyten, besonders schön für *Anisothecium Hookeri* diesen Fall belegen. In der Tat ist mir aus der Süßwassertierwelt kein Fall bekannt, der die circumantarktischen Teile dieser Kontinente verknüpfte. Hingegen ist die seltsame Disjunktion zwischen dem tropischen Südamerika und Madagaskar, die bekannte Ravenala-Disjunktion, auch in der Süßwasserfauna vertreten durch das Vorkommen der *Cypris ravenala* Brehm die mit der madagassischen *C. Decaryi* Gauth. nächstverwand ist (vgl. Brehm, 1934, wo auch auf Seite 77 Beispiele aus anderen Organismengruppen nach Irmscher zitiert sind).

Zum Schluss möchte ich darauf verweisen, dass West- und Nordaustralien so gut wie eine terra—oder in unserem Falle besser aqua—incognita darstellen. Aber auch wenn einmal aus diesen Gebieten hinlängliche Süßwasseruntersuchungen

vorliegen werden, dürfte sich zeigen, dass zwischen diesen Gebieten und dem südöstlichen Australien ein grosser faunistischer Gegensatz besteht, der nicht etwa nur ökologisch bedingt ist. Die in der älteren Literatur vorliegende Angabe vom Vorkommen eines *Diaptomus* aus der *orientalis*-Gruppe in Queensland, lässt erwarten, dass diese Teile Australiens der Diaptomidenregion angehören, so dass also Australien ebenso wie Südamerika aus zwei heterogenen Teilen hinsichtlich seiner Süsswasserfauna zusammengesetzt ist, eine Erscheinung, die auch mit Wegeners Anschauungen im besten Einklang steht.

SUMMARY

This review gives a survey of endemic organisms of the fresh-water fauna of the circumpolar regions, which have geologically, but not ecologically, separate habitats. A number of examples has been selected which indicate that various animals occur in approximately the same geographical distribution areas as Irmscher established for his plants, although the insufficiency of our knowledge of large regions (e.g. Western and Northern Australia, New Guinea, the west of the Argentine Republic) does not allow of the zoological maps being so complete as the botanical. The similarity of the animal and plant results, however, may perhaps support Wegener's theory.

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ANHANG

Erst nach Drucklegung dieses Artikels bemerkte ich, dass für die soeben erwähnte, so seltene Süd-Disjunktion Südamerika und Südafrika—tropische Disjunktionen dieser Art gibt es ja genug—vielleicht doch auch Fälle aus der Süßwasserfauna vorliegen. Es sei auf das Vorkommen der von Sars in Südafrika entdeckten *Cypridopsis spinifera* hingewiesen, die Kliever kurzem für Uruguay nachgewiesen hat. (Klie, W. "Süßwasser-Ostrakoden aus Uruguay", *Arch. Hydrobiol. Plankt.* 29, 1935, 294.) Noch auffälliger als diese Speciesdisjunktion ist aber der Fall, den die noch problematische Gattung *Godetella* Del. bildet. Diese Harpacticidengattung wurde von Delachaux für die von ihm im Huaronsee in Peru (5140 m.) entdeckte Art *G. Kummli* aufgestellt, wobei der Verfasser bemerkte, dass die von Richard aus Argentinien beschriebene *Mesochra Deitersi* auch zu der Gattung *Godetella* zu stellen sei. Chappuis hat nun in seiner Arbeit "Zoologische Ergebnisse einer Reise nach Bonaire" (*Zool. Jahrb. Abt. System.* 64, 1933, 396 ff.) zu dieser Gattung kritisch Stellung genommen und kommt dabei zu dem Ergebnis, dass *Godetella* mit der Gattung *Cletocamptus* identisch sei. Die Trennung wurde von Delachaux besonders mit Rücksicht auf die basale Verschmelzung der Furkalborsten bei *Cletocamptus* vorgenommen, die bei *Godetella* fehlt. Indem Chappuis diesem Merkmal keine besondere Bedeutung beimisst, plaidiert er für eine Vereinigung der Gattungen *Cletocamptus* und *Godetella*. Da aber auch das ökologische Verhalten und die geographische Verbreitung diese beiden Gattungen trennen—*Cletocamptus* ist ein fast ganz auf die nördliche Halbkugel beschränkter Salzwasserbewohner, *Godetella* ein so ziemlich auf die südliche Halbkugel beschränkter Süßwasserbewohner—möchte ich doch zu bedenken geben, ob die Trennung der beiden Gattungen nicht besser aufrechtzuerhalten wäre. Aber auch, wenn man dem nicht zustimmt, bilden die *Godetella*-arten innerhalb der weiter gefassten Gattung *Cletocamptus* auch bei Chappuis eine zusammengehörige Gruppe, deren nachstehend notierte geographische Verbreitung dem oben erwähnten Disjunktionsbild entspricht:

Godetella Kummli Del. Peruanische Anden. *G. Deitersi* (Rich) Patagonien, Argentina. Sollte *G. Brehmi* Kiefer und *G. Bermudae* Willey mit *Deitersi* identisch sein, was ziemlich sicher ist, so würde das Areal dieser Art nach Centralamerika reichen, da *Brehmi* in der Laguna Peten in Guatemala gefunden wurde und *Bermudae* auf den Bermudas.

G. trichotus Kiefer bei Kapstadt.

Während also die von Irmscher auf Seite 203 des zweiten Bandes seines wiederholt zitierten Werkes behandelten Arten von Patagonien bis zu den Kerguelen reichen, so wie *Pseudo-boeckella brevicaudata* unter unseren Beispielen, nicht aber den afrikanischen Kontinent erreichen, hätten wir bei *Godetella* einen Fall, der den afrikanischen und südamerikanischen Kontinent verbindet, allerdings durch den weiten Vorstoß nach Norden auf der amerikanischen Seite nicht mehr gut als circumantarktisch bezeichnet werden kann.

SELECTIVITY CONTROLLING THE CENTRAL-PERIPHERAL RELATIONS IN THE NERVOUS SYSTEM

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I. INTRODUCTION

FROM the results of a series of experiments carried out in 1921-2, the conclusion has been reached that the relationship between the central nervous system on the one hand and the sense organs and muscles on the other is not based simply upon typical structural interconnections of an innate pattern, possibly modifiable to a certain degree as a result of trial and error (conditioning, learning), but that the facts are such as to necessitate the invoking of some principle of highly *specific* relations, other than structural, controlling the response of each individual peripheral organ.

This principle of specific relations linking the periphery with the centers has been called the "resonance principle". It was first derived from observations on the "homologous response" of supernumerary transplanted limbs in *Amphibia* (Weiss, 1922). G. Hertwig, in 1926, repeated and confirmed the experimental

results. In all these earlier experiments, fully developed functional limbs had been used as grafts. In 1925, Detwiler reported the phenomenon of homologous response in limbs which had been transplanted as buds in the embryonic stage, and analogous observations were mentioned by several authors incidental to other types of work (Brandt, 1925).

Periodically the progress in the field has been reviewed in short articles (P.W.¹ 1928 *b*, 1929 *b*, 1931 *c*), and most recently by De Silva and Ellis (1934).

A considerable mass of experimental data obtained in recent years, but not yet published, will be utilized in the following account in addition to published records.

II. THE PHENOMENON OF HOMOLOGOUS RESPONSE

The original experiments consisted of the transplantation of fully developed whole limbs in free-swimming salamander larvae (*Salamandra maculosa*); more recently various species of *Amblystoma* were used. The operation has been described in detail (P.W. 1923 *d*, 1929 *a*). The limbs to be grafted were taken from either the same or from another individual. In order to assure their innervation from part of the limb nerve plexus, they were grafted into the vicinity of a normal limb at a distance sufficient to insure their complete mechanical independence of each other. Save for this general restriction, there was no further uniformity observed in their insertion and orientation.

Innervation for the grafts was provided by the severing of some branches of the limb plexus, part of the transected fibers subsequently regenerating into the deserted nerve tracts of the new limbs. A more detailed description of the mode of innervation will be given below.

The loss of nerves in the original limb caused by the partial interruption of its nerve supply was in no case heavy enough to entail visible defects in its motility. The grafts, paralyzed during the first weeks, showed the first signs of recovering function at some time between two and four weeks after the transplantation, varying with the age of the host and with the season. However, not until several weeks after the earliest movements appeared did the function reach full strength.

The very first movements observable show the characteristic traits of "homologous function". For convenience, we shall proceed here with a description of the activity of the grafts as it is found after sufficient time has been allowed for complete functional restoration, leaving the antecedent stages of partial function to a later chapter. The fully recovered motility of the re-innervated grafts was found to obey strictly the following rules (we designate as *T* the transplanted limb, and as *O* the original limb in whose nerve plexus *T* participates) (P.W. 1924 *b*, p. 637; 1928 *a*, p. 47):

During spontaneous and reflex activity each muscle of *T* contracts always and exclusively at the same time (synchronous response) and with the same relative degree of intensity (syndynamic response) as the corresponding muscle of the same

¹ These initials refer throughout to publications by the author.

name in *O*; biceps with biceps, anconaeus with anconaeus, and so forth. Muscles of the same name will henceforth be called "synonymous" muscles.

In the original experiments, performed on small animals, the muscular contractions were determined from the movements they produced; at that time, no claim was made of having observed individual muscles, as an obviously misinformed critic has tried to imply (Versluys 1927, 1928; see, however, the reply P.W. 1928 *c*, 1929 *c*). Since 1929, however, studies on an adult frog (Verzár & P.W. 1930) and on large sized axolotls (P.W. 1936 *b*) have actually made it possible to analyze without difficulty the activity of the individual muscles, and the results have been found to be in perfect agreement with the statements in the earlier papers. Throughout, the above rule, describing the phenomenon of "homologous" (*i.e.* synchronous and syndynamic) response of synonymous muscles has been found to be substantiated for each individual limb muscle tested.

The rule of homologous response which from its inflexibility deserves to be proclaimed a law, has been derived from a great variety of experimental results as their only conceivable common denominator. Conversely, all the various experimental results can be predicted on a single assumption, namely the general validity of that rule. A detailed discussion of the main conditions under which homologous response has been observed follows. The data presented in the account have been collected from experiments performed at various times, partly still unpublished. They are based on 180 successful cases of limb transplantations studied in detail, and almost 200 cases of muscle transplantation.

(1) HOMOLOGOUS RESPONSE IN THE MOTOR FIELD

Homologous response between limbs of identical laterality. In these experiments, a left forelimb was transplanted into the vicinity of another left forelimb. If both *O* and *T* can be moved freely, their movements represent exact duplications of each other with perfect synchronism and congruity. Neither was ever found to move without the other. Specifically, identity of synchronous movements in *O* and *T* has been observed for the following movements: flexion, extension, pronation and supination of forearm; adduction, abduction, dorsal flexion, plantar flexion of hand; abduction, adduction, flexion and extension of whole digits; flexion and extension of single phalanges. In addition to these, homologous movements of the upper arms—abduction, adduction, elevation, depression, inward and outward rotation—were observed in all those cases in which *T* had been left in possession of a shoulder joint and part of its girdle, while upper arms inserted directly into the body were immobilized.

The study of motion pictures, permitting an accurate analysis of the movements, has revealed that the synchronism between synonymous muscles in *O* and *T* is so precise that even the intermediate phases of each movement, traceable through the successive frames of the film, representing periods of about 0.05 sec. each, are identical. Once the re-innervation of the grafts has become complete, not only the time of their contraction, but also the intensity of contraction, is the same for the synonymous muscles in *O* and *T*. While the tensions of the contracting muscles

could not be recorded directly, the positions of the joints during and at the end of a movement served as indicators for the degree of contraction. Comparing *O* and *T* on this basis, it was found that the angular positions of elbow, wrist and of corresponding fingers, at any given moment, were as nearly congruous as it was possible to ascertain from study of the motion pictures.

This is the picture invariably found when both limbs are free to move, as, for instance, in animals suspended freely in air or in water, exhibiting struggling or wiping movements, or during swimming. If, however, one of the limbs encounters resistance of some sort, temporarily barring its movements, the phenomenon of homologous response changes in appearance, though not in character. The synonymous muscles in *O* and *T* still contract simultaneously, but while in the free limb the contractions are isotonic, producing movement, in the impeded limb they are isometric, producing tension. This is most commonly observed in the act of walking, where *T*, not reaching the ground, can move unhindered, while *O* takes part in the propelling of the body (P.W. 1924 *b*, p. 639): the initial contractions, creating merely increased pressure against the ground in *O*, appear in the unresisted *T* from their very onset as movement. The outcome is that in this act *T* seems to be slightly ahead of *O*, and in its movements it actually is, although the muscular contractions are strictly synchronous in both. Since the movements of *T* thus serve to signal all concealed isometric contractions in *O*, supernumerary limbs can be used as convenient indicators in an analysis of muscular activities during locomotion. The fact that homologous contractions in the muscles of a synonymous pair can be made to appear as isometric in one and isotonic in the other, has also proved valuable in the study of myotatic (proprioceptive) reflexes (Verzár & P.W. 1930, see below).

If one pays due attention to the fact just mentioned, one realizes that it is altogether incorrect to speak of "homologous movements", as has become the use. What is really homologous, is the contraction of the muscles, whereas the effects of these contractions, whether movement or increased tension, depend on the external circumstances which often differ with respect to the two limbs. It is needless to dwell longer on this point, since the evidence accumulated in the following pages will make it plain that in the phenomenon of homologous response only the muscular contractions are relevant, and not their effects.

If the transplants have been inserted in their normal anatomical orientation (dorso-dorsally in the terminology of Harrison, 1918), the synchronous movements of *O* and *T* are parallel, resembling the strokes of a rowing crew. In the majority of cases studied, however, the transplants had been rotated into an abnormal orientation, making them useless for the body. Irrespective of the orientation of *T*, it was always the synonymous muscles in *O* and *T* that contracted simultaneously. For instance, an elbow flexion in *O* was invariably accompanied by an elbow flexion in *T*, regardless of whether or not this accessory elbow flexion was of any avail to the body. In transplants rotated toward the back or head or belly, it certainly was not: in swimming, the direction of their strokes deviated from the backward strokes of the normal limbs by the same amount for which they had been rotated; in walking, they could not reach the ground, and during wiping movements of the normal

forelimbs, they idly beat the air. It is beyond doubt, therefore, that no adaptive value can be conceded to the phenomenon of homologous response (P.W. 1924 *b*, p. 638; P.W., 1928 *a*, p. 9).

Homologous response between limbs of opposite laterality. When a right forelimb is grafted near a left forelimb, again the synonymous muscles in both contract simultaneously. Since right and left limbs are, however, in their anatomy, mirror images of each other, the movements produced by homologous contractions in *O* and *T* are also mirror images. In a right limb transplanted to the left side in such a way that its dorso-ventral axis is not inverted (dorso-dorsally), the elbow points cephalad instead of caudad as in a normal limb. Hence, the contraction of, for example, the muscles extending the forearm, appears in *O* as a stroke backward, but in *T* as a stroke forward. Similarly, all other movements of *O* and *T* are symmetrical with respect to a transverse plane. If such a *T* can reach the ground, it may be of considerable hindrance to the animal, inasmuch as it produces all the locomotor reactions of *O* in exactly the reverse sense, *O* and *T* thereby cancelling each other's efforts. Although *O* and *T*, moving against each other, become frequently entangled, especially in cases where they are in close proximity, no change in this behavior has ever occurred. The contention of Bethe and Fischer (1931, p. 1119) that under conditions like these a dissociation between *O* and *T* might possibly result, has, therefore, not been substantiated by the facts.

Interchange of limbs of opposite laterality. By means of a modification of the preceding set of experiments the absence of adaptive features in the phenomenon of homologous response could be demonstrated still more strikingly. First, in a number of specimens of the type described in the previous section and in which the reverse functioning—reverse from the standpoint of the body—of the grafts had been ascertained, the normal left limbs were amputated, leaving only the grafts at the site. The idea was the following: as long as the normal pattern of impulses is being delivered to *O*, the *T* of opposite laterality is bound to operate in the reverse sense because, as we have seen, a dissociation between *O* and *T* is impossible. But, after the removal of the normally functioning *O*, might not some corrective adjustments take place in the central patterns tending to make the now solitary *T* co-operate with, instead of counteracting, the activities of the body? Nothing of the sort occurred. *T* continued to function in the same reverse manner, as at the time when *O* was still present.

The next step was to remove *O* even before *T* was transplanted so that the two would never co-exist, and *T* have no opportunity, therefore, to become directly associated with the normal *O*. The experiments involved the mutual exchange of the two forelimbs (P.W. 1935 *d*). A right limb was substituted for the removed normal left limb, and a left limb was substituted for the right limb, the grafts again oriented dorso-dorsally so that their palmar surfaces were in contact with the ground as under normal conditions. After the nerves had regenerated, when these limbs began to function, it was evident at once that their actions, though well co-ordinated in themselves, were strictly reverse with regard to the aims of the body. In other words, in each graft at any given instant those muscles contracted which would have

contracted, at the same instant, in a limb of the normal laterality in the same place. The function of the graft is exactly as if it were homologous with the function of an imaginary normal limb which may be visualized as being virtually present on the site and as performing its normal role in locomotion. But since the graft is a mirror image of the visionary normal limb which should be in its place, since the flexors of its elbow are on its posterior border instead of on its anterior border and the extensors on the anterior instead of on the posterior side, the fact that those muscles always contract which are meant to contract in the normal limbs leads to an effect precisely the reverse of that which would suit the body. Thus, if an animal intends to walk forward, its interchanged forelimbs actually move it backward. This type of functioning, perfectly absurd from the standpoint of the body, has never ceased nor even changed during the several months of observation, extended beyond the metamorphosis of the animals.

Some important conclusions can be drawn from this set of experiments. First and principally we learn that the association of a given muscle of *T* with the synonymous muscle of *O*, as found in supernumerary limbs, is not at all the salient feature of the phenomenon of homologous response. For the phenomenon appears unfailingly, even if an *O* with which to associate is no longer actually present. This means that what had seemed to be an association of *O* and *T* in the cases where both were present is in reality merely an expression of the fact that they both depend in an identical way upon a common third. This third is—let us call it, for the moment, by an anthropomorphic term—the central order.

In a normal limb the contraction of a muscle discloses to the observer that this muscle has, at that instant, been called into action by the centers. Therefore, in all our experiments with supernumerary limbs, *O* could serve as an indicator to point out which muscles the centers had called into activity in any given reaction. Wherever we could avail ourselves of an *O* to play this role, we have found that a central call destined for a particular muscle *A* is executed peripherally not only by the original muscle *A*, but by every additional synonymous muscle *A*, present in the district, as well. Both sets of muscles, the ones in *O* and the ones in *T*, are reached by common commands which, for some peculiar reason, they seem to be forced to obey in an identical manner; hence the homologous contractions.

It is evident that the ultimate commands are not given out by the centers in terms of limb function, such as "thrust", "flexion", "grasp", or the like, but are already fragmented into the elementary calls for the individual muscles to participate in the movement. Provided the muscles called for are in their typical positions, a proper effect suitable to the whole results, as intended. But if they are located or grouped or inserted abnormally, their stereotyped obedience to calls that have been devised for normal relations manifests itself in such absurd functional effects as were observed in the experiments of this series. Concerning the situation in mammals, see below.

We conclude, then, that what we have called homologous response is not at all due to a direct coupling of *T* with *O*, or of their respective central representatives, but to a peculiar linkage between the centers and either of them. *T* duplicates rather

than imitates the activity of *O*, and the presence of a normal *O* would be entirely immaterial in the study of homologous response were it not that it serves as a convenient indicator as to the contents of the central commands.

A correct formulation of the phenomenon of homologous response would therefore read as follows: whenever a call to act is given out from a spinal district for a particular muscle *A* (as indicated by the response of the muscle *A* in the normal limb, if such is present), all muscles *A*, present in the same district of innervation respond. If the call is for a muscle *B*, all muscles *B* react, and so forth. This formulation contains nothing hypothetical; it merely avoids the unwarranted allusion to a non-existing imitation of *O* by *T*.

Another point brought out convincingly by the continued reversed functioning of interchanged limbs of opposite laterality is the surprising lack of flexibility and adaptability of muscular co-ordination in Amphibia. The disturbance of locomotion created by the operation, although very serious, failed to call forth a central adjustment that would have either changed the pattern of discharges to conform to the needs of the organism, or have suppressed them altogether. For a further discussion of this point see p. 525.

Transplantation of multiple limbs. In 1930, a frog with two functional supernumerary limbs was described (Verzár & Weiss, 1930). Since then, a number of salamanders have been equipped with two or even three supernumerary forelimbs, the multiple supernumeraries being either of identical or of different laterality (P.W. 1936 *b*). As a rule it is impossible to secure adequate nerve supply for more than two transplants in addition to the normal limb, which limits the number of transplants possible. Besides, nothing could be gained by an indefinite increase in their number. The phenomenon of homologous response repeats itself in each additional limb: if there are two or three *T* present in addition to *O*, again the synonymous muscles in all these three or four limbs are found to contract simultaneously. Oriented in various ways and crowded into a rather narrowly circumscribed locality, as they are, these limbs become continually entangled with each other to such an extent that this behavior strikes the observer as no less absurd than the reversed walking described in the preceding section.

Transplantation of limb fragments. In 1923 an experiment was reported in which the distal portion of an arm, including forearm and hand, was substituted for a whole arm; the wrist, in this case, always moved at such times and in such directions as it would have moved had it still formed part of a whole arm (P.W. 1923 *e*, 1924 *b*, p. 643). This showed that neither the integrity of the limb nor the completeness of its muscular endowment is prerequisite to obtaining homologous response. Each muscle responds to its call independently, regardless of whether or not the other muscles of the limb are present. Later experiments (P.W. 1931 *a*) with transplanted single muscles brought corresponding results (see below). Transplanted limb fragments containing only a partial set of muscles duplicate the function of the exactly corresponding portion of the normal arm.

Similarly, in transplants of whole limbs in which owing to post-operative accidents the proximal muscles have degenerated or failed to receive innervation, the

distal parts as the only functional ones, exhibit homologous response with the precisely corresponding sections of *O*.

The condition under which the fractional nature of the phenomenon of homologous response can be ascertained most easily is the stage of incipient re-innervation of the transplants, which we have omitted from consideration above. Not all the muscles receive their innervation at the same time, and apparently it is largely a matter of chance which ones come first. In some cases the earliest reactions appeared in the shoulder, but it is equally frequent occurrence that the elbow or the fingers move first. These first movements, appearing between two and four weeks after the transplantation, already conform to the principle of homologous response, so far as is possible to decide by direct observation unaided by accurate registration of muscular activities. The first observable activity of *T* may, for instance, consist of a weak flexion of the elbow, always occurring simultaneously with an elbow flexion of *O*; for a few days, these elbow flexions may be the only movements in *T*, and since the extensors are still paralyzed, the forearm is brought back into its original position after each flexion only by virtue of the elasticity of the antagonists and their tendons. Several days later additional movements may appear, for instance, in the digits, identical with the movements of the corresponding digits of *O*. Thus, the number of muscles resuming activity increases in steps, until eventually, within about two more weeks, the entire limb functions vigorously in all its joints. As mentioned above, there exists no definite rule as to the order of re-innervation, and the non-adaptive character of the phenomenon of homologous response can scarcely be questioned in view of this manner of functional restoration in haphazard and unrelated fragments, where the agonists function while the antagonists are still paralyzed, and fingers move with the elbow still inactive. Only in the end, after every muscle has received nervous connections, do we obtain, by piecing together those fragments, the picture of a smooth and co-ordinated movement.

In no stage does the function of the transplant as a whole appear to be of any import. Thus in the restoration of function there is seen no logic other than that embodied in the principle of homologous response.

We wish to stress the fact that, in contrast to some results on adult animals to be described later, non-homologous mass contractions were never observed in these larval limbs, not even during the earliest stages of functional recovery.

Regeneration of double limbs. Hyperdactylism and other forms of duplication can be produced in the regenerating limbs of larval and adult urodeles in various ways (P.W. 1924 *a, c*). The duplicated elements, if functional, invariably exhibit homologous response.

If a transplanted limb is fused lengthwise with a normal limb and the distal part is amputated within the common stump, a double and intimately fused regenerate with two free hands may develop (P.W. 1924 *c*), the identical parts of which again show homologous response. For instance, in the composite regenerate developing from two limbs coalesced along their dorsal surfaces, the dorsal muscles lie in the center and the forearm contains plantar muscles on both free sides. Accordingly,

the two rows of fingers crowning the common trunk always move in opposite directions (P.W. 1924 *b*, p. 642).

Homologous response between limbs of different size. In the transplantation experiments thus far reported donors and hosts had been of approximately the same age and size. Recently, however, a few experiments were performed to determine the behavior of grafts considerably smaller than the limbs of the host (P.W. 1936 *b*). In a number of salamanders forelimbs were removed from young and small specimens and grafted near forelimbs of much older and larger animals, the ratio between their volumes often being as much as 1 : 10. Again in these cases, notwithstanding their difference in size, both limbs gave homologous response, and the intensities of the synchronous contractions of synonymous muscles were approximately in proportion to their respective size. This conclusion was derived from a study of the motion pictures, revealing that in every phase of a movement the angles of corresponding joints of the large *O* and the small *T* were nearly identical.

Homologous response between forelimbs and hindlimbs. If in salamanders a forelimb is transplanted into the innervation district of a hindlimb, both show homologous response (P.W. 1924 *b*). In this case it is not the synonymous but the homologous muscles that respond simultaneously. While no study of the individual muscles could be made, it was still possible to identify muscle groups by the movements they produced, and to ascertain the homology involved. Thus, the following major movements of *O* and *T* appeared always paired (*O* being a hindlimb, *T* being a forelimb): extension of knee—extension of elbow; flexion of knee—flexion of elbow; dorsal flexion of foot—dorsal flexion of hand; plantar flexion of foot—plantar flexion of hand; abduction of foot—abduction of hand; adduction of foot—adduction of hand; movements of the toes—corresponding movements of the fingers.

We see, then, that a call sent out by the spinal center for a certain muscle is answered not only by all the synonymous muscles, but also by all the homologous muscles present in the district. In the face of these clear-cut experimental facts the homology between fore- and hindlimbs in the urodele Amphibia can hardly be questioned. Inasmuch as the limbs acted as they did, they were obviously to this extent homologous. We mention this because from the criticism raised on phylogenetic grounds by Versluys (1927) it seemed as if there were an inconsistency between the homologous response of these limbs in the actual experiments and the theoretical expectations based on comparative phylogenetic studies.¹

The reciprocal experiment—transplantation of hindlimbs to the shoulder—has also been performed, but fewer functional results were obtained, apparently because of the failure to secure adequate nerve supply for the transplants (P.W. 1924 *b*, p. 670).

In anuran Amphibia forelimbs grafted in the place of hindlimbs have been

¹ Versluys published his criticism in 1927. After a reply by the author (P.W. 1928 *c*), he reiterated his stand (Versluys, 1928). Since Versluys, in both his papers, had made use of distorted citations, the author considered his part in the discussion as closed and further discussion as quite unprofitable (P.W. 1929 *c*). Versluys, however, continued the controversy, turning against Boeke (Versluys, 1930), and finally Boeke (1930), emphasizing the futility of a further discussion, closed the series.

observed to move, but no study of the type and degree of movements has been made (Gräper, 1924).

It would naturally be of great interest to know the range of divergence in specificity within which homologous muscles can be substituted for each other. Such work, performed on higher vertebrates, might even help to establish homologies on an experimental instead of on a speculative basis. The interesting work of Barron (1934), dealing with the function of forelimbs partially innervated from hindlimb nerves in the rat is still too complex in its conditions to warrant evaluation in one or the other direction.

Homologous response in heteroplastic transplants. G. Hertwig (1926) was the first to show that homologous response is not confined to limbs of the host's own species. The limb of a newt, *Triton*, transplanted to a salamander, *Salamandra*, showed perfect homologous response with the nearby salamander limb. Similarly, homologous response was obtained after transplanting limbs between different species within the salamanders (*Amblystoma mexicanum* and *A. punctatum*) (P.W. 1936 *b*). It may be mentioned in this connection that the transplantation of embryonic limb buds between embryos of different species (Harrison, 1935 *a*) also yields functional limbs; the fact that the size of these limbs is often out of proportion to the body is not inimical to their co-ordinated functioning, according to our results described above (p. 502).

We realize, therefore, that not only the homologous muscles of fore- and hindlimbs of the same species, but also the homologous muscles of different related species, possess in common properties which enable each to respond to central calls of the other.

Transplantation of embryonic limb buds. While differentiated and wholly functional limbs have been used for all the transplantation experiments described thus far, Detwiler and others (Detwiler, 1925; Detwiler & Carpenter, 1929; Detwiler & McKennon, 1930) have clearly demonstrated that essentially the same results can be obtained if the limbs are grafted as buds in the embryonic stage. When function is later assumed, the limbs which have developed from the grafts and the normal nearby limbs display homologous response provided they are both innervated from the same spinal district. We shall return to these experiments in a later section.

Homologous response in congenital duplications. Congenital duplications of limbs or their parts, such as are occasionally observed in many higher vertebrates including man, are generally assumed to be caused by accidental disturbances in the early embryonic history of the limb blastema (Przibram, 1921; Bonnevie, 1934). Consequently, they are experiments executed by nature, and as such may serve as substitutes where direct experimentation is not practicable, as in the higher vertebrates where it has not yet been possible to apply the experimental tests to which the Amphibia have been so easily amenable. Cases of supernumerary formations produced by accidents during embryogenesis have, for the most part, attracted only anatomical interest, and their functional examination has been deplorably poor and fragmentary.

Of special interest is a human case in which the phenomenon of homologous response has been ascertained. A girl with a congenital malformation of the left arm and hand, first described by Halverson and Amatruda (1935), was re-examined (P.W. 1935 *e*). Radius and thumb were absent and their place was taken by a second ulna and a second set of the ulnar three fingers, aligned in reverse order. The hand, therefore, carried seven mobile and well-differentiated fingers, which were, beginning with the most lateral one (n =normal, s =supernumerary): $n\ 5$, $n\ 4$, $n\ 3$, $n\ 2$, $s\ 3$, $s\ 4$, $s\ 5$. In voluntary and reflex action there was strict synchronism between $n\ 5$ and $s\ 5$, $n\ 4$ and $s\ 4$, $n\ 3$ and $s\ 3$, and the corresponding fingers moved in the same sense. Thus far no sure sign of dissociability between identical muscles has been detected, the pairs of synonymous muscles in this human hand working together as consistently as did the pairs of synonymous muscles in duplicated amphibian limbs, but it would be unsafe to venture predictions as to the possible dissociation resulting from continued training.¹

(2) HOMOLOGOUS RESPONSE IN THE SENSORY FIELD

Just as in the motor field each muscle was found to respond properly to the orders issued by the centers even after it had been dislocated, so in the sensory field the centers could be shown to recognize and identify afferent messages coming from muscles in arbitrary abnormal locations. Thus, a proprioceptive message arriving from a supernumerary biceps muscle, for instance, is precisely recognized centrally as being sent from a biceps and from no other muscle.

This fact has been disclosed by the study of myotatic reflexes in supernumerary limbs. Certain muscles, when stretched passively, receive from the centers a reflex impulse to contract, and this remains fairly well circumscribed and confined to the very same muscle whose stretching had started it off (Sherrington, 1929; Hoffmann, 1934). When a muscle A has been stretched, a motor discharge is presently released by the centers for muscle A , and when a muscle B has been stretched, the motor impulse is for B . Now, in our experimental animals with the duplicate limbs, every motor discharge for muscle A activates both muscles A , the one in O and the one in T , and similarly, every discharge for B activates both muscles B . This double response has the advantage that it allows the untouched limb to register immediately as movement those contractions which in the muscles held under passive stretch appear merely as increased tension and which it would require elaborate methods to detect. While the limb subjected to passive stretch cannot move, its duplicate, being perfectly free, serves as an indicator, revealing by the type of its movement whether or not the centers have correctly identified the exact origin of any proprioceptive message started from the other limb.

By this method it was found (Verzár & P.W. 1930) that messages coming from muscles of the supernumerary limb, abnormally located, functionally idling and

¹ A recent re-examination of the case, 8 months after the former study, revealed that with proper attention and effort homologous fingers could be moved individually; but in subconscious actions, as well as in stages of fatigue and distraction, homologous fingers were still used in association (P. W. and T. C. Ruch, *Proc. Soc. exp. Biol. N.Y.* 34, 1936).

quite atypically innervated (P.W. 1931 *b*), are identified as correctly as are those coming from the normal limb.

After stretching the muscle *B* in *T*, one obtains a motor response which is perfectly definite and correct, appearing in all the muscles *B*. Stretching a supernumerary *A* makes the two muscles *A* contract reflexly, and so on for each type of muscle from which proprioceptive reflexes can be elicited. By the accuracy and correctness of their motor reactions the centers show that they possess means to identify the precise muscular origin of a proprioceptive excitation regardless of the possible abnormality of location, functional significance and nerve supply of the muscle. On the other hand, apparently no distinction is made between multiple synonymous muscles, since the excitations coming from a muscle in *O* yield the same effects as those coming from the synonymous muscle in *T*.

The first crude observations of this kind were made on the muscles of the hand in salamanders (P.W. 1923 *b*, 1924 *b*). A more thorough study, aided by motion pictures, followed several years later in an adult frog possessing two supernumerary free limbs on one shoulder (Verzár & P.W. 1930). The fact that every motor response in this area occurred in triplicate, made it possible to demonstrate that proprioceptive excitations coming from extensors, flexors, pronators, supinators of the forearm; dorsal flexors, plantar flexors, adductors, abductors of the wrist; flexors, adductors, abductors of digits, or even of individual phalanges, were each one correctly identified by the centers, no matter from which limb of the limb triplet they were issued. From the further fact that the degree of each motor response corresponded to the amount of stretch, it follows that in addition to the kind of muscle the amount of stimulus applied to it also was correctly apprehended by the centers. Concerning the nerve supply in this case, see P.W. 1931 *b* and p. 510 below.

Recent experiments on large specimens of salamanders have shown, with regard to the hand and finger muscles, the same reactions as had been obtained on the frog; the elbow muscles in the salamanders proved, however, less apt to produce myotatic reflexes, so that here not the whole number of muscles listed above could be tested.

In the human case with congenital duplication referred to above, the situation was found to be very much the same as in the Amphibia. The girl, prevented from controlling her abnormal hand visually, identified the supernumerary fingers *s* 3, *s* 4 and *s* 5, as well as the normal fingers *n* 3, *n* 4 and *n* 5, when moved separately, as middle finger, ring finger and little finger, respectively. This means that an excitation from *s* 3 evokes the same sensation as an excitation from *n* 3; and similarly, *s* 4 and *n* 4, and *s* 5 and *n* 5 were perceived as identical (Halverson and Amatruda, 1935; P.W. 1935 *e*).

In conclusion, we find in the relationship between sensory organs and centers the same peculiarity which we had found to characterize the relation between the centers and muscles: namely, that their mutual correspondence remains unaltered despite arbitrary variations in the location of the peripheral organs. This statement, so far as it concerns the sensory phenomena, must, for the time being, remain limited to the proprioceptive field, as the only one for which conclusive evidence has thus far been obtained.

(3) HOMOLOGOUS RESPONSE AFTER CENTRAL OPERATIONS

Homologous response persists in animals after they have been decerebrated by a cut across the medulla oblongata (P.W. 1936*b*). This definitely proves that the phenomenon of homologous response is based on properties of the *spinal* mechanism and that the intervention of centers higher than spinal has nothing to do with it. An attempt to penetrate by means of operative methods into the spinal limb center itself will be reported in a later section.

(4) CONCLUSIONS

As the one common and constant feature embracing the considerable variety of findings reported in the preceding sections, we recognize the fact that some specific and unmodifiable relation or correspondence exists between each individual muscle and its central representation. This specific relation insures that every central message will unfailingly reach the addressee of the right name. Under the most diversified conditions the individual muscle has been observed to respond invariably in accordance with its *name*, biceps with biceps, anconaeus with anconaeus, flexor carpi radialis with flexor carpi radialis, and so forth. And nothing but the name, neither the location of the muscle with respect to the whole functional system, nor the usefulness of the limb to the body as a whole proved to have any weight.

Of course, the name of the muscle stands simply as a symbol for the entity of individual characteristics that distinguish various muscles from each other. While it has been our habit to regard most of those individual characteristics as being partly of anatomical and mechanical significance—shape, relation to the skeleton, texture and arrangement of fibers, etc.—partly of a general physiological concern—slow, fast, tonic, tetanic—the phenomenon of homologous response has, for the first time, opened our eyes to the fact that the name of each muscle represents something much more fundamental and essential: namely, *a constitutional specificity determining its relationship to the nervous system, constant and selective for each individual muscle, identical for synonymous and homologous muscles, but differing critically for different and non-homologous muscles.*

No longer can we, therefore, look upon the muscular system as merely a mass of flesh, cast into this or that shape, attached in this or that manner, but throughout of the same making, and with its relations to the nervous system partly pre-established by typical connections and partly controlled by a principle of functional adjustments (learning) in which the appropriateness to the body of the results is all that counts; quite to the contrary, we find the muscular system composed of as many discrete and distinct individualities as there are non-homologous muscles, each muscle endowed with a differential and non-adaptive specificity which prevails over all other concerns such as anatomical arrangement and functional significance. And it cannot be stressed strongly enough that this discriminating specificity has been proved to belong really to the individual muscle, and that one would fail to do justice to the facts by conceding it merely to larger groups of muscles in a more general way; for instance, to “flexors” and “extensors”.

The fact that each muscle possesses certain personal characteristics distinguishing it from all other muscles has been known since the work of Schiefferdecker (1911, 1913), in which histological and cytological differences between different muscles were clearly demonstrated. But it has not occurred to anyone to suspect that these microscopic differences may signify a more intrinsic and specific differential, instrumental in controlling the neuro-muscular relations.

III. INTERPRETATION OF HOMOLOGOUS RESPONSE

Every attempt at explaining how a muscle, by virtue of some individually specific property residing in it, may determine its eventual relationship to the nervous system must take into account three possibilities:

- (1) that specific messages reaching the centers from the muscles by way of the sensory nerves may somehow be the agents involved ("learning" hypothesis);
- (2) that some selective power may guide particular nerves to their proper muscles during the process of re-innervation as well as during first development (morphological selectivity);
- (3) that the admission of excitations from the centers to the periphery may be controlled by some resonance-like selectivity (physiological selectivity).

These three possibilities will now be discussed successively.

(1) CAN SENSORY CONTROL ACCOUNT FOR HOMOLOGOUS RESPONSE?

The first possibility mentioned would come under the general heading of "learning, conditioning, central adjustment", and the like; our idea of the neurological basis of these processes is vague enough to admit easily one more phenomenon unprovided for in the conventional neurological schemes. In fact, on several occasions the attempt has been made to shelve the phenomenon of homologous response by relegating it to a demonstration of just what queer things the centers can "learn" to do. It has been overlooked, however, that the two most significant features of conditioning or learning phenomena were found to be missing in homologous response, namely, modifiability and adaptive value for the body as a whole (P.W. 1924*b*, p. 639; 1928*a*, p. 9, 1931*c*). Many striking examples to support this assertion can be found above (pp. 498-501).

More concise than the general learning idea is the contention that, the functional irrelevance of the phenomenon being granted, still the significant clues concerning the position and connection of the muscles of a supernumerary limb might be furnished to the centers by proprioceptive excitations from those limbs. Other than proprioceptive control could be ignored, since many of the transplants were beyond the limits of the visual field of the animal, and tactile stimulation was absent in those grafts which did not actually make contact with the ground. That proprioceptive control, however, is equally unconcerned with the appearance of the phenomenon of homologous response has been shown by the following crucial experiments.

Exploratory experiments to determine the degree to which motor patterns in *Amphibia* depend upon proprioceptive control revealed that this dependence is

usually considerably overrated. In both the toad and the salamander the motor functions were found to remain essentially unimpaired in limbs from which the sensory control had been eliminated by extirpation of the dorsal nerve roots and the spinal ganglia (P.W. 1934 c, 1936 a).

If, then, into the vicinity of such a de-afferented, but motile, limb an extra limb was transplanted, sharing in the now purely motor nerve plexus, again the typical phenomenon of homologous response appeared in the two limbs, both of which were completely anaesthetic (P.W. 1935 c).

These results exclude definitely the possibility that homologous response may be due to secondary central adjustments effected under the direction of sensory control. So we turn to the second possibility.

(2) CAN MORPHOLOGICAL SELECTIVITY ACCOUNT FOR HOMOLOGOUS RESPONSE?

Morphological selectivity of a kind that could offer an explanation of the phenomenon of homologous response would require that, in addition to the muscular differential, a corresponding differential of specificities should exist in the nerve fibers, and that during the process of re-innervation each muscle would attract and admit exclusively its proper and predestined type of nerve fibers, rejecting connections with fibers of a specificity other than its own.

There is twofold evidence on hand to disprove the assumption of this type of selectivity: evidence of a general nature gathered from the study of nerve regeneration and evidence obtained directly from the experimental cases showing homologous response.

(a) *The problem of specificity in nerve regeneration*

Re-innervation of limbs transplanted in post-embryonic life takes place by the sprouting out of regenerating nerve fibers from the severed host nerves into the grafts (P.W. 1923 b, 1924 b). Therefore, the general rules of nerve regeneration can be applied. Nerve regeneration differs from nerve ontogenesis in two main respects: first, in the extensive branching of the individual axons, due presumably to mechanical conditions near the cut surface (P.W. 1934 a, p. 440), and, second, in the distances which must be bridged by the fibers on their way to the end-organs which are immensely greater in the developed form than in the embryo where innervated organ and innervating source lie close together (Harrison, 1935 b, p. 172). Regenerating fibers must grow down the whole length to the peripheral organs; for, it has been proved, not only for mammalian nerves (cf. Boeke, 1921), but also for amphibian nerves (Williams, 1930; Speidel, 1935), that severed fibers are unable to restore their integrity by mere fusion of the proximal with the distal fragment; the distal fragment degenerates and must be replaced by outgrowth from the central stump.

This being the case, it would require very powerful, and at the same time very specific, directive agencies, in order to cause each type of nerve fiber eventually to reach the proper type of muscle. All the evidence available stands overwhelmingly against the existence of such specific factors. Experiments on tissue cultures of

nerves have, thus far, failed to reveal any tangible factor orienting the growth of the nerve fibers other than the mechanical (microscopical or ultramicroscopical) structure of the medium (P.W. 1934 *a*). Chemical agents proved effective only indirectly, to the extent to which they influence the structural pattern of the medium. Electrical agents were altogether ineffective (P.W. 1934 *a*; Karssen & Sager, 1934; against Ingvar, 1920, see P.W. 1934 *a*, p. 426). Although it is an undeniable fact that growing organs and other "neurotropic" sources exert directive effects on developing nerve fibers (Tello, 1923), this can be readily explained on the mechanical theory of orientation (P.W. 1932 *b*, p. 334, 1934 *a*, p. 435), and several competent authors concur in this interpretation (Detwiler & Van Dyke, 1934; Harrison, 1935 *b*). If, however, the guiding principle is mechanical, there is very little room left for strict specificity.

Moreover, lack of specificity in the directing factors has often been demonstrated experimentally: nerves grow readily into strange organs, both in the embryo (Braus, 1905; Hoadley, 1925; Detwiler, 1928, 1930; Nicholas, 1929, 1933) and *in vitro* (P.W. 1934 *a*, p. 443) when left to themselves. An almost unlimited number of examples could be added to illustrate the unspecificity of nerve connections in cases where nerves have been forced to grow into strange tissues. The successful substitution of one motor nerve for another is common practice in human surgery (Stookey, 1922). Crossings between somatic and autonomic nerves have been performed with functional results (Langley and Anderson, 1904). Motor nerves can be made to connect with sensory organs and sensory nerves can be made to form motor connections with muscles (Boeke, 1917); in the latter case, the transmission of excitation from the sensory nerve to the muscle over the strange connection has been demonstrated (P.W. 1934 *b*). Peripheral nerves can be forced to end in the spinal cord (P.W. 1932 *a*), and sensory roots, which normally end in the spinal cord, can be connected with muscles (P.W. 1935 *a*).

This choice of examples would seem sufficient to suggest that, inasmuch as even the most disparate types of nerve fibers—somatic and autonomic, central and peripheral, afferent and efferent—can vicariously innervate each other's end areas, there is no basis for the assumption that a particular muscle may admit only a particular sort of motor fiber.

While the idea of morphological selectivity in nerve regeneration can thus already be refuted on general grounds, a thorough examination of the mode of re-innervation of transplanted limbs has greatly helped to strengthen the point.

(b) *The neurotization of transplanted limbs*

Since only severed nerve fibers seem able to produce regenerating branches, some of the normal limb nerves had to be transected, in order to furnish a source of innervation for the transplants. In the earlier experiments (P.W. 1923 *b*, 1924 *b*) the accidental interruption of some plexus nerves by the insertion of the transplant was found to provide for a sufficient supply. Any part of the plexus could, by excessive branching of its fibers, refill the transplants. The nerves concerned were in many cases some which had formerly been connected with a limited group of muscles

only; nevertheless, in the transplants, they made connections with the full set of limb muscles present, thereby demonstrating their non-selectivity (P.W. 1924 b, p. 648).

It was merely by peripheral branching that the nerves took care of the additional load of peripheral muscles. No increased outgrowth of motor fibers from the spinal cord could be detected (P.W. 1928 a, p. 18). This observation corroborates fully the analogous fact established for embryonic urodeles by Detwiler: overloading the periphery with muscles does not reflect on the quantitative development of the spinal cord (Detwiler, 1933¹).

In a more recent series of experiments, a controlled nerve supply was chosen for the transplants at the time of the operation (P.W. 1934 b, 1936 b). Of the three nerves chiefly composing the forelimb plexus in salamanders, *i.e.* the 3rd, 4th and 5th, the 5th is generally the weakest and its transection causes no appreciable functional defect in the limb. This nerve was then assigned to the transplants. It has been stated that in *Amblystoma* the 5th nerve normally supplies the muscles of the hand and the wrist only (Detwiler & Carpenter, 1929; Carpenter, 1934). This statement has been challenged by Nicholas & Barron (1935) who, by stimulating the 5th nerve with electric currents, could demonstrate that it contributed to the innervation of muscles over the whole length of the arm. Carpenter (1934) claims that even in regeneration the 5th nerve fails to innervate other than distal muscles. This contention, however, is clearly contradicted by our cases in which the 5th nerve, inserted into the transplanted limbs, made connections with all the limb muscles present.

A count of the number of nerve fibers leaving the cord revealed that the addition of one or two transplanted limbs to the periphery had caused no appreciable, if any, increase in the number of fibers proximal to the plexus. The augmentation necessary for the innervation of the accessory limbs is produced purely by the branching of the existing fibers during their regeneration. The growing of branches into the transplants continues until the limbs are filled to their capacity with fibers, the final number of fiber branches present in each limb being strikingly close to normal. This quantitative study, together with a similar one made previously on a frog with two supernumerary limbs (P.W. 1931 b), corroborates the estimates made in an earlier paper (P.W. 1928 a), and the combined evidence leads to the following statements:

(1) The transplanted limbs are fully innervated. (2) This innervation is effected entirely by the peripheral branching of pre-existing nerve fibers, no new motor fibers or collaterals being sent out from the spinal cord toward the grafts. (3) The number of cells at the level of the spinal cord innervating the grafts shows no alteration. (4) Therefore, as far as can be revealed by microscopic study, the spinal cord and the proximal parts of the motor nerves have been left unaffected in their morphological aspects by the adding of one, two, or three limbs to their peripheral load.

When we consider that a comparatively small number of fibers produces a large number of branches; that the branches grow out independently; and that no

¹ *Biological Reviews.*

selectivity guides them to their final destination; we cannot escape the inference that branches of the same fibers may often come to end on different muscles. This inference, in agreement with the current notions on nerve regeneration, is certainly more than mere conjecture. On the other hand, no direct substantiation of it has yet been forthcoming, and for this reason we refrain, for the time being, from emphasizing it as strongly as had been done in the earlier papers up to the year 1928, when the resonance principle was postulated chiefly on the strength of this point. We shall return to the matter later.

Leaving the numerical relations and turning to the peripheral distribution of the nerve fibers in the transplanted limbs, we find the earlier anatomical studies (P.W. 1924 *b*, 1928 *a*, 1931 *b*) and the more recent studies (P.W. 1934 *b*, 1936 *b*) in which the anatomical method of tracing the nerves was supplemented by electrical stimulation in full support of each other. Crucial cases for the stimulation tests were those in which plexus nerves had branched into both the original limb and the transplant. Stimulation of such a common trunk should be expected to yield identical responses in both limbs, if the branches had connected selectively with synonymous muscles in *O* and *T*. However, this did not as a rule occur, quite different and unrelated muscles contracting in *O* and *T*, offering thereby the most direct experimental test for the fact that synonymous muscles are by no means innervated by common nerves. In many cases the regenerating nerve fibers, before entering the graft, intertwined irregularly in a chaotic net-like neuroma and from this the graft drew its supply. This is the most conspicuous illustration of the random character of the innervation.¹

Adding to these results the evidence obtained from the muscle transplantation experiments reported below, we can safely conclude that no strict morphological selectivity that could serve to explain the phenomenon of homologous response governs the re-innervation of the grafts. Therefore, by exclusion of the other two, the third possible explanation, that of some physiological selectivity, has been brought to the fore.

IV. THE RESONANCE PRINCIPLE

A common solution seemed possible on the assumption that the central nervous system and the non-nervous periphery entertain their mutual correspondence by means of some sort of sending-receiving mechanism, specific for each individual muscle. One might, for instance, conceive of a spinal limb center as being endowed with a capacity for discharging as many different modes or forms of impulses as there are different muscles in the limb, an appropriately specific impulse for every one of them. The muscle, on the other hand, would possess the power to respond selectively, each one to its proper impulse and to no other. We realize that if, then, the central impulses for a limb muscle were circularized in the whole limb district, a mechanism of this making would insure that every call be answered by the correct

¹ In a recent crucial experiment the transplanted limbs were assigned for their innervation a branch of the inferior brachial nerve (ulnar) cut at the elbow level so as to contain exclusively fibers for wrist and finger muscles. Transplants supplied in this manner exhibited homologous response in all muscles, including those of shoulder and upper arm.

muscle, even though the latter may have been displaced, re-innervated by strange nerves, and prevented from sending informative messages back to the centers. Two or more synonymous muscles, present in the same district, would, as a result of their identical selectivity, respond to the call simultaneously, in other words display homologous response.

In this form the resonance principle of nervous control was originally promulgated (P.W. 1923 *c*, 1924 *b*). As the main innovation it postulated that the conventional switchboard scheme be replaced by a less geometrical principle, essentially *dual* in character: on the periphery a set of selective individual receivers, facing a central system of diffusing, non-apportioned, but specific emissions. Stated in this general form, the original resonance principle is still the only concept that has been able to offer an adequate and consistent explanation of the phenomenon of homologous response. In the details, however, especially those regarding the delimitation of what should be considered as the specific "periphery", the course of later developments has necessitated certain revisions, which were, for the first time, communicated to the American Society of Zoologists in 1934 (P.W. 1934 *b*, 1935 *a*, p. 137).

The term "resonance principle" has been chosen merely for the sake of illustration, to symbolize the general analogy to the selective responsiveness observed in physical and chemical "resonance" systems. No further implication as to the particular type of "resonance" involved should be associated with the term. It is noteworthy (P.W. 1928 *a*, p. 88) that a similar mechanism is found in the hormone systems, where localized effects are due to the specific selectivity of the reacting peripheral tissues toward the circulating glandular substances. Thus, the basic principles employed in the two principal correlation systems of the body, in the neural and in the humoral system, reveal certain striking parallels (Lugaro, 1924; Parker, 1932). But we wish to attribute no further significance to this fact in the present connection.

(1) THE PERIPHERAL SPECIFICITIES

In comparing the traditional theory with the resonance theory, one discovers as the main point of divergence that the former assumed the activation of a muscle by the centers to be a one-sided act, requiring merely a central discharge, whereas the latter demonstrates that the response is conditioned from both ends of the line, in a sense, inasmuch as something peripheral which can ultimately be traced to the individuality of each muscle controls the admission or non-admission of central discharges. This something, amounting to a very specific peripheral selectivity, although determined by the muscle, need not necessarily be expected to be located in the muscle itself. Only in the original outline of the resonance principle (P.W. 1923 *c*, 1924 *b*) was the muscle itself assumed to possess the power to analyze the central discharges and to select its proper call out of the full set dispatched to the periphery. But already in 1928 this view was abandoned in favor of the assumption that the actual selectors were parts of the peripheral nervous system, presumably the nervous terminals. It was assumed that each muscle, by virtue of its own specificity, appropriately specifies the nerve endings, converting them from in-

different into selective receivers specifically adapted to its own use (P.W. 1928 a, p. 64). This process, previously called "specification", will henceforth, with a more suggestive term, be called "modulation" (P.W. 1934 b); the nerve acquires a specific "modulus".

The question as to how far down a nerve fiber its modulation may extend was left open. While preference was given to the view that it may be limited to the terminals, the possibility that the whole fiber may gradually be modulated was suggested as an alternative (P.W. 1928 a, p. 66; 1931 a, pp. 605 and 656; 1931 c, p. 1216; 1935 a, p. 137). As a crucial experiment to decide the question, the following was outlined in 1928 (P.W. 1928 a, p. 124). Taking for granted that the action potentials of a nerve are intimately connected with, and infallibly indicative of, its state of activity, one should expect, in case the central activity of a given limb district were really discharged uniformly over all the motor nerve fibers of the limb, to be analyzed only more peripherally, that then at any moment all the fibers would be found in the same state of either activity or inactivity.

The experiment was actually performed by Wiersma (1931) on frogs during reflex activity. The results failed to substantiate the above expectation. The various hindlimb nerves tested were not active simultaneously, and action potentials appeared only in those nerves whose muscles actually contracted in the reflex. These results were confirmed in a recent study of action potentials during reflex activity by the present writer (unpublished). They force us to conclude that the normal peripheral nerves carry only the impulses destined for their own effectors, and nothing more. In other words, at the moment when the excitations leave the spinal cord and enter the peripheral nerves, the act of specific selection, responsible for the muscle-correct apportioning of the componental impulses, is already over. This obviously settles the above question; for, if the selection takes place within the centers, and is yet, as we have demonstrated, in accord with the muscle on the peripheral end of the line, it is clear that the process of modulation, extending centrad from the muscle, must have continued beyond the nervous terminal, over the whole length of the motor fiber, down to the spinal cord (P.W. 1934 b; 1935 a, p. 137, footnote). Wiersma has arrived at this same logical conclusion.

At first it seemed difficult to reconcile this fact with the other fact, mentioned before, that different muscles innervated from common motor neurones can be activated independently. As will be shown later, this difficulty has been overcome, at least in principle. Partly on the strength of the evidence coming from the action-potential experiments, partly on other grounds, a decided shift has occurred in the emphasis as to the extent of modulation. It now seems inevitable to assume that *modulation embraces the entire "motor unit"* in Sherrington's (1929) terms, including the motor neurone. This view, though regularly mentioned as a possibility to be kept in mind, certainly failed to receive adequate attention in the earlier papers on the resonance principle and was not elaborated into details until recently (P.W. 1934 b).

It seems logical to assume that, as the motor nerve fibers are modulated by their muscles, so the sensory nerve fibers are modulated by their end-organs. As a result

we arrive at the following idea of modulation. Specific influences emanating from the non-nervous receptor and effector organs, and individually distinctive for each of them, induce in the erstwhile indifferent nerve fibers terminating there appropriately specific changes. A fiber ending on a gastrocnemius muscle would be converted into a gastrocnemius-specific fiber, admitting only gastrocnemius-specific impulses; a fiber connecting with a semitendinosus muscle would acquire semitendinosus selectivity; and so forth. A method of tracing the process of modulation will be reported below. Experiments to use action current registration in its study are under way.

The modulation of a nerve fiber by its muscle may plausibly be supposed to be a biochemical effect. This may mean either of two things: (1) the muscle fiber may impregnate the nerve fiber with a specific substance different for each muscle, such substance gradually diffusing centripetally; or (2) the change in the nerve fiber may be of the nature of immunological processes, the muscle protoplasm sensitizing the nerve protoplasm in a specific manner after both have come in contact. Now, neither the existence of different emanations from different muscles, nor the presence of a serological organ differential among protoplasms of different muscles has thus far been demonstrated, presumably partly because it has not been searched for, partly because its detection may be beyond the limits of our methods up to date. Accordingly, all attempts at defining more concretely what modulation may consist of are at present bound to remain pure speculation.

A nerve fiber not yet connected with an end-organ is regarded to be non-selective and to admit any mode or form of central impulse. When such a nerve fiber, then, enters into connection with, for example, a muscle *A*, a certain modulus α , which we may visualize as a specific biochemical substance or as a definite ratio of substances and which must be instrumental in the process of propagation, is differentiated in the fiber as a result of the impregnating or sensitizing action of the muscle *A*. Nerve fibers connecting with a muscle *B* receive a modulus β , and so forth. If, during later life, a nerve fiber is detached from its original muscle and connected with a different muscle, as in our limb transplantation experiments, it abandons its old modulus and acquires the new one. Whether a fiber loses its old modulus after mere disconnection from the old periphery, or whether contact with a new periphery is required to supersede the old modulus remains to be seen; experiments to decide the question are under way. Concerning the reversibility of modulation, see below.

It is conceivable that in the nerve fiber, concomitant with its modulation, various morphological and physiological changes may take place which could serve as indicators of the gradual functional differentiation; for instance, changes in average fiber diameter, threshold or latent period. Although differences of this sort are known to exist among different nerves, no experiments have been performed to determine to what extent they may reflect influences exerted upon the nerves by the non-nervous peripheral end-organs and effectors. Prior to such an examination no prediction can be made in regard to possible visible changes associated with the modulation of the nerve fiber.

With regard to the nervous terminals, we are, however, in a more fortunate position. Their qualitative differentiation has been proved clearly to depend upon the character of the non-nervous tissues on which they are formed. Boeke (1917) found that sensory fibers connected with muscles form ramifications of the motor type, and that the terminations of motor nerve fibers invading a sensory periphery resemble sensory endings. Thus the character of the ending is determined by the periphery rather than by the nerve fiber. That the determining action on the part of the periphery is highly specific follows from experiments of Dijkstra (1933). Limb nerves, penetrating into flaps of skin transplanted from the duck's bill to the leg, were found to develop the sensory endings characteristic of the bill and normally absent in limbs. Such specific and determining influences exerted by one organ upon another fall into the general class of phenomena known in embryology as "inductions" (see P.W. 1935 *b*, p. 650). In view of these facts, demonstrating effects of the non-nervous periphery on the morphological character of the nerve fibers invading it, the assumption of a functional specification, as postulated by our experiments, may lose some of its strangeness.

By virtue of what we have called modulation, the non-nervous periphery takes possession of the peripheral nerve fibers to such an extent that eventually they have become more truly part of the periphery than of the central system from which they had grown out. On general grounds, it seems conceivable and even likely that modulation does not remain confined to the peripheral neurons, but from there continues into the central fiber tracts of the white matter, appropriating ("assimilating") the latter to the periphery.

However obscure the nature of modulation may still be, there can be no doubt as to the real existence of such a process. It was recognized for the first time in the course of muscle transplantation experiments in adult animals (P.W. 1930 *b*), and the evidence has been strengthened by recent results which will be outlined below.

(a) Transplantation of individual muscles

The resonance principle has ascribed to each individual muscle a specific constitution, controlling its relation to the nervous system. In order to put this contention to an additional and more direct test, it was deemed desirable to transplant single supernumerary muscles instead of whole limbs or limb fragments. A method was devised by which whole muscles in adult toads could be successfully transplanted and re-innervated at will (P.W. 1930 *a*). The first extensive series of experiments performed with this method brought results which, on the whole, confirmed the predictions of the resonance principle (P.W. 1930 *b*, 1931 *a*).

In metamorphosed young toads various supernumerary hindlimb muscles were transplanted to the back. Degeneration of the muscles could be prevented by keeping them under stretch, tension apparently being favorable for the survival of cross-striated muscle fibers (P.W. 1933). For nerve supply some limb nerve was chosen which was known to have had no previous connection with that sort of muscle, *e.g.* the nerve from an antagonist muscle, thus obviating the objection of morphological selectivity (see p. 511). About four weeks after the operation, the first functional

connections were found to be established; in four more weeks most of the muscle fibers had received innervation. Then the activity of the transplants was recorded and compared with the normal limb muscles during spontaneous activity and in reflexes elicited by adequate, mostly mechanical, stimulation. In each animal simultaneous kymograph tracings were made of the contractions of three muscles: (1) the transplant, (2) the synonomous muscle of the normal limb of the same side, (3) some other limb muscle for control. From the records thus obtained it was evident that the tracings of (1) and (2) ran essentially parallel. If the transplant was, for instance, a gastrocnemius, its contractions coincided with the contractions of the normal gastrocnemius; if it was a semitendinosus, they coincided with the normal semitendinosus; and so forth. The intensity and duration of these synchronous contractions of synonomous muscles were also found to correspond fairly well.

Two cases out of twenty-eight behaved exceptionally, the function of the transplanted muscle being not identifiable with that of its synonomous fellow (P.W. 1931 *a* p. 639). The experiments have been repeated since and extended on a larger scale, thereby uncovering more exceptions. After a survey of the whole series, comprising more than 100 pertinent cases, one is left with the impression that, the older the animals used for the experiments, the more inexplicable the results. Finally, in full grown adults the results described for younger animals seem almost irreproducible. Since no rigorous statistical test has so far been accorded to these cases, no final conclusion can be drawn. But the results seem to suggest that with increasing age the modulated state of the nerves becomes irreversibly fixed. An alternative explanation is that in the older nerves it would have required more time to supersede the original moduli than was allowed to the experiments. Undeniably the situation needs further investigation.

More definite clues concerning the problem of modulation of nerves can be gathered, however, from the following observations (P.W. 1930 *b*, p. 366; 1931 *a*, p. 632). While the majority of the fibers of a transplanted muscle, several weeks after its re-innervation, contract in specific association with the synonomous muscle, many transplants were found in which part of the fibers contracted indiscriminately, that is, together with any limb muscle in action. These unspecific responses indicate that the fibers concerned lack specific selectivity. They are in a despecified and not yet respecified condition and, therefore, admit all sorts of impulses. By comparing the various cases on the basis of specificity of response and time elapsed since the operation, it became apparent that during the later stages of recovery the proportion of transplants containing unspecifically responding units was notably smaller (P.W. 1931 *a*, p. 654). It must be added, that the number of cases (twenty-six) on which this comparison has been based, is not nearly sufficient to permit a final statement. But if correct, the observation would lend itself to a plausible explanation. The more time had elapsed since the reconnection of the nerves with their new muscles, the more of the despecified nerve fibers would have acquired the new moduli.

These experiments, then, contained the first indication that modulation is something proceeding in time, and, under the given conditions, proceeding comparatively

slowly. Even several months after the transplantation some units were still recorded as responding unspecifically.

Recently a method has been developed making it possible to follow directly the transformation of unspecific into specific responses (unpublished). In young metamorphosed toads the left hindleg was denervated and connected with one of the nerves of the right limb. Consequently, the left leg served, in a sense, as a supernumerary leg of the right side and should be expected to exhibit homologous response with the normal right leg. As a matter of fact, homologous response eventually appeared. But between the period of immobility and the final stage of homologous response a well-marked intermediate stage of unspecific contractions was observed. In this transitional stage the left limb showed only one single type of reaction, namely a general contraction of all the re-innervated muscles. Upon mechanical stimulation of almost any part of the body, the limb responded invariably by assuming a rigid extension with a characteristic tremor. Not until many weeks later did some orderly and distinct movements appear, homologous with the movements of the right limb, and emerging from the earlier background of indiscriminate mass contraction. In our terms, this means that after an earlier stage with absence of selectivity, hence indiscriminate response, in the despecified nerve fibers, gradually remodulation of the fibers from their new muscles occurs, resulting in the emergence of selective responses.

In these experiments, modulation could therefore be visualized directly. It proved to be a very slow process, requiring many weeks. With this suitable method now at hand, an exact quantitative study is planned in the hope that the time characteristics of the process may reveal something about its nature.

It is remarkable that the transitory unspecific stage found in the toad has not been encountered in the salamander experiments. The difference can be explained, it seems, by the fact that the salamanders were in the larval stage, while the toads were metamorphosed. A process which in the older and metamorphosed animals with their more consolidated tissues may take considerable time, can well be supposed to proceed in the young and plastic larval tissues with sufficient speed to escape our attention.

(b) Mononeuronal connection between receptors and effectors

Still another method has been devised that seems to open a promising approach to the study of peripheral specificity and modulation, namely, the establishment of a direct line of communication between sense organs and muscles by means of common neurons with complete elimination of the centers. Instead of letting the peripheral sensory excitations discharge into the spinal cord, and allowing the motor excitations in turn to be picked up from it, an attempt was made to couple sensory senders and motor receivers directly. The attempt was successful (P.W. 1935 *a*). In adult toads dorsal nerve roots were severed from the spinal cord and inserted into transplanted muscles. Thus, the sensory neurons, still connected peripherally with their end-organs in skin and muscles, are forced to discharge proximally immediately into a muscle. In a number of cases, the dorsal root fibers

actually achieved functional connections with the muscle, so that electrical stimulation of the nerve produced a regular twitch. In contrast to the positive effects of electrical stimulation, however, the adequate stimulation of the nerve from its sensory end-organs (mechanical and chemical stimulation) remained entirely ineffective on the muscle. One may hesitate to accept this negative result as final, pending a controlled check by action potential registration of the excitations sent from the sensory periphery toward the muscle. But according to our conception the result could have been anticipated. At the time of the operation, the sensory neurones were in possession of a modulus corresponding to their peripheral end-organs. After the operation, the muscle attached to their proximal ends begins to spread its own modulating effect. Consequently, the nerve fibers are exposed to two different modulating agents, acting from both ends, and ultimately presumably resulting in a lengthwise subdivision of each neurone into two parts of different specificity. If we make the further fundamental assumption that excitation can be propagated in a nerve fiber only as far as the elements of its conductive mechanism are all of identical specificity (see below), it would follow that propagation of an excitation started from the sensory end would be blocked at the region of transition from the sensory-specific part of the fiber to its motor-specific part. An alternative explanation would be that an adequate excitation started from a sensory end-organ contains something in its make-up that renders it unfit to excite a muscle (P.W. 1929 *b*; Verzář & P.W. 1930; P.W. 1935 *a*). Further experiments on these problems are under way.

As mentioned above, a certain complication arises from the fact that in nerve regeneration a number of peripheral fiber branches arising from a common neurone may be connected with different muscles or different sensory end-organs, and consequently be subjected to different forms of modulation. At the points of bifurcation the different processes would meet, and in order to account for the fact that the different muscles at the ends of such branches can be activated alternately, and that sensory messages coming over such split fibers can still be discriminated, one would have to postulate that the two or more modulations converging upon the common stem of the fiber can continue into the stem and coexist therein without interfering with each other. The common stem of a motor fiber would remain permanently accessible to more than one type of central excitation, indeed to as many as there are different muscles connected with the fibre, and not until in the ultimate peripheral branches would the excitations appear definitively filtered out for their respective end-organs. This conjecture, if seemingly far-fetched, yet appears to be the only one that can account for the observations.

On the sensory side, a similar problem has been known to exist for some time. The branching of sensory fibers to different end-organs has been demonstrated morphologically (Dogiel, 1904; Botezat, 1909; Ohmori, 1924) and physiologically (Hoagland, 1932; Barron and Matthews, 1935), and in order to explain the fact that sensations are nevertheless kept from being confused, one might have to adopt some scheme similar to that outlined here for the motor field.

(c) The nature of nerve specificity

While we do not hesitate to admit our complete ignorance regarding the nature of the selective state introduced into the nerve fiber by the process of modulation, we may yet try to approach the problem by the method of exclusion. We can eliminate from further consideration a few more or less obvious suggestions, for the reason that they proved to be inconsistent with the facts. In this group belong all the attempts to explain the selectivity in terms of specific time characteristics, either specific frequencies, or specific time forms of the impulse as expressed in the intensity-duration curve of liminal stimuli.

Only in the first publications on the resonance principle were specific frequencies regarded as a possible instrument of selectivity (P.W. 1923 *c*, 1924 *b*). As early as 1928, however (P.W. 1928 *a*, pp. 103-5), mainly under the influence of the demonstration by Adrian (1926 *a, b*) and Adrian & Zotterman (1926 *a, b*), of the fluctuating rhythms of action potentials, this idea was rejected. The main objection comes from the variable frequency of the discharges in individual nerve fibers (Adrian & Bronk, 1929; Adrian, 1932) which is evidently correlated with the intensity rather than with the specificity of their effects. Direct attempts to obtain selective responses to different rhythms of electrical stimulation also failed (Achelis, 1930). It is deplorable that notwithstanding repeated clear statements to the contrary, some authors still indulge in identifying the resonance principle with the idea of specific frequencies.

The suggestion that the theory of isochronism in the sense of Lapique (1926) may account for the phenomenon of homologous response, has been injected into the discussion of the resonance principle by v. Brücke (1929). According to Lapique the transmission of excitations from nerve to muscle or from nerve to nerve is limited to those units which have nearly identical time parameters (chronaxie). Undeniably this theory, inasmuch as it assumes the admission of excitations to a given nervous or muscular unit to be controlled by functional properties rather than by stereotyped connections, bears a certain fundamental resemblance to our resonance principle. The possibility, however, that there may be a more intimate relationship between both, which several years ago may have seemed not at all remote, has faded completely in view of the development of the problem of isochronism in recent years.

It is not only beyond the scope of this article to enter into a discussion of the technical subtleties involved in the controversy pro and contra the theory of isochronism (Rushton, 1930, 1932, 1935¹; Lapique, 1934, 1935¹), but it is also quite unnecessary, for two reasons: first, heterochronism has been asserted to differentiate predominantly between general groups of muscles, particularly between flexors and extensors as mass (Bourguignon, 1923), whereas no strict and infallible discrimination between the individual muscles within a flexor or extensor or otherwise synergic group seems possible on the basis of their chronaxial values. This lack of reliable specificity, muscle by muscle, renders the whole conception inadequate to serve as a basis for the resonance principle. Secondly, even those chronaxial values which were

¹ *Biological Reviews.*

supposed to distinguish the muscles and nerves, if not individually, at least in groups, were found to fluctuate considerably, as long as the central connections of the nerves are left intact (*chronaxie de subordination*, L. and M. Lapicque, 1929, 1934). Now, it is evident that this variability is quite the opposite of what we had to postulate for the specific parameters of the individual muscles. The existence in each muscle of a constant, inalienable and unalterable constitutional characteristic determining its response to the centers, as proved by our experiments, is irreconcilable with the assumption that the response may be controlled by such an unstable fluctuating character as *chronaxie* has come to be; even less so since "*chronaxie de subordination*" has been shown to supersede the more stable "*chronaxie de constitution*" of the isolated muscle. Had the *chronaxie* of the muscle been demonstrated to reflect on the *chronaxie* of its nerve, it would have been possible to admit a general compatibility between the resonance principle and the theory of isochronism; since, however, exactly the opposite has been found, namely, that the centers affect the *chronaxie* of the nerve, it becomes obvious that the two theories can have no intimate bearing on each other.

Moreover, direct measurements on our toads of *chronaxie* values in the transplanted muscles, the synonymous normal muscles and their respective nerves failed to show correlations that would parallel their homologous responses (experiments done by the author in collaboration with Mme Lapicque, see P.W. 1931c, p. 1216).

Time specificity being excluded from our choice of possible mechanisms, chemical specificity of some sort challenges our interest.

Again, such chemical specifications as have thus far become known as being instrumental in neuro-muscular transmission (Loewi, 1934; Cannon, 1934) are so crude and generalized that they cannot account for the considerable variety of neuro-muscular specificities revealed by our experiments. The specific affinity of one type of end-organ or muscle toward acetylcholin and of another type toward adrenalin or sympathin may be regarded by some as at least a faint hint as to the sort of principle into which the problem of peripheral specificity may possibly resolve itself in the future. But a single substance acting in a general manner on all muscles can certainly not be the carrier of those specific effects which discriminate between the individual muscles, unless that substance be capable of assuming as many specifically different forms or modifications as there are muscles.

It must also be born in mind that the domain of those chemoneuric affinities is chiefly, if not wholly, the autonomic system, dealing with muscles of the involuntary type, while our experiments have been exclusively concerned with skeletal muscles.

Although none of the chemical associates of nervous activity uncovered heretofore meets our demands in the point of variety, none of our results, on the other hand, seems to be inimical to attributing some role in the ultimate transmission from nerve ending to muscle to a substance like acetylcholin, as has been suggested for the skeletal muscle by Dale & Feldberg (1934). The transmission from nerve to muscle may well be conceived as unspecific, provided only the preceding transmission within the nerve is specific. In fact the "unspecific" contractions observed in transplanted toad muscles (p. 516) indicate that the muscle, while having the

modulating power by which to render its nerve selective, apparently possesses no selective power of its own toward the excitations; which seems to support the idea of an entirely unspecific transmission from nerve to muscle.

On the whole, there is no end to speculation, but as yet little solid ground on which to identify "resonance" in our terms with a particular sort of chemical affinity. Resonance phenomena on a chemical basis are conceivable and have been subject to mathematical treatment (Raschevsky, 1930).

Tentatively, we may follow the hypothesis formulated above, contending that

- (1) a certain sensitive biochemical compound of the nerve fiber is instrumentally involved in the mechanism of propagation of the impulse by bioelectrical currents;
- (2) this compound is specifically sensitized (modulated) by contact of the nerve fibre with a specific muscle or end-organ;
- (3) the propagation of excitation can only take place between compounds of identical specificity but not between compounds of different specificity.

Since a single axone under normal conditions is connected, as a rule, with only one type of end-organ, subjected to only one type of modulation, and therefore is of identical specificity throughout, propagation in it proceeds unhindered, offering us the aspect of all-or-none character of conduction. The all-or-none principle in its recognized limits (Adrian, 1933), then, seems no longer irreconcilable with the resonance principle, as it had seemed in the earlier discussions (P.W. 1927*a*; 1928*a*, p. 114). But when a fiber is composed of tandem parts of different specificity, as is presumably the case in our mono-neuronal receptor-effector connections (P.W. 1935*a*), excitations may be extinguished at the point of transition as if in a narcotic bloc.

(2) THE CENTRAL ACTION SYSTEM

The modulated peripheral neurones find themselves confronted and in counterplay with a central system of activities into which the sensory excitations discharge and from which the motor neurones pick up selectively their respective components. We may call this central system the central action system.

What we know of it beyond the obvious fact of its location in the gray matter of the cord, is very little indeed. We know that it contains the innate dynamic patterns for motor co-ordination, and that it circularizes the specific calls for the individual muscles provided for in these patterns. The autonomous existence of these spinal patterns as an intrinsic property of the spinal cord (Brown, 1914) is suggested by their considerable independence of afferent control (P.W. 1936*a*), as well as by our experiments with interchanged limbs (P.W. 1935*d*; see above, p. 498). It is obvious, furthermore, that each spinal district consists of two symmetrical halves, discharging separately into their respective peripheries, the left half into the left limb and the right half into the right limb, neither of them communicating directly with the periphery belonging to the other; if this were not so, the asymmetrical activation of the two sides of the body would be impossible, since the synonymous muscles of both sides are of identical specificity (P.W. 1931*a*; see also above, p. 498).

Anteriorly and posteriorly the emitting district for a limb seems to be bounded by the limits of the segments contributing to the limb plexus. Some experiments of Detwiler (1920, 1922), Detwiler & Carpenter (1929) and Detwiler & McKennon (1930) lead inevitably to this conclusion. These authors found that no limb could function properly unless it received at least part of its innervation from the normal limb segments of the cord. No spontaneous and co-ordinated movements were ever obtained in limbs innervated from the trunk segments of the cord (see also Ruud, 1929), although some ill-defined twitches could apparently be secured in response to direct stimulation of the limb. According to these results it seems that the impulses specific for limb muscles are produced within the limb segments of the cord and normally do not spread beyond the limits of these segments (P.W. 1924 *b*, p. 667; 1928 *a*, p. 81). The delimitation of the local sending systems for other organs than the limbs is an empirical task for future work. It is conceivable that, comparable to the situation in embryology (P.W. 1935 *b*, pp. 654–6), the functional districts are represented by local fields of activity, whose boundaries are maintained in a dynamic way by the mutual interference of neighbouring areas.

In this connection some results call for our attention which at first sight seem not to fit into the general scheme. In contrast to the immobility of limbs innervated from trunk segments, movements of greater extent have been described in limbs innervated from cranial nerves. Nicholas (1929) and Detwiler (1930) found that limbs transplanted into gill segments moved in association with gular or gill movements. The question of whether or not this functional association between limbs and gills lasts indefinitely, answered contradictingly by the two authors, is of less concern to us than the mere fact that limb muscles did apparently respond to central emissions patterned for gill muscles. According to Detwiler (1936) these grafted limbs displayed neither the differentiated pattern nor the variety of normal limb movements, each individual case being capable of only a single and stereotyped movement, back and forth, accompanying the elevation and depression of the gills.

Several explanations of this result are possible (P.W. 1931 *a*, p. 655): (1) The limbs, having been transplanted in the embryonic stage as buds, may have been invaded by local muscles, derivatives of the gill muscles. The movements of the limbs would thus have been effected by genuine gill muscles, hence naturally associated with gill movements as true homologous response. (2) The movements may have been effected by muscles whose nerves had, for some reason or other, failed to undergo modulation and, therefore, were open to elevator and depressor impulses (see below). Additional experiments are needed to decide between these two possibilities.

In one particular instance, a critical re-investigation has already led to some clarification. Nicholas (1933) had stated that a limb grafted in the embryonic stage into the orbit of an enucleated eye, later moves in co-ordination with the opposite eye. One suspects that embryonic eye muscles may have penetrated into the limb bud and produced the later movements. In order to exclude this possibility, the experiments were recently repeated (P.W. 1936 *b*), using developed limbs which are no longer exposed to extensive muscle shifts. In larval salamanders a developed fore-

limb with its shoulder girdle was grafted into the socket of the enucleated eye (twenty-seven cases). Where the eye muscles had been left in place they established new insertions on the base of the graft and could accordingly move the limb as a whole, as they would have moved the eyeball. The limb muscles proper, however, remained inactive and no distinct movements occurred in any of the grafts. In a few cases, a faint twitching of some of the limb muscles was seen to accompany the feeding reaction of the jaws and gills, so feeble that it was not perceivable except under the microscope. This was a perfectly stereotyped reaction remaining constant for each individual, though involving different muscles in different individuals; in this respect it reminds us of Detwiler's cases mentioned above. It is most likely that we are dealing here with unspecific responses of a small number of aberrant units which have failed to become modulated, comparable to what we had found in the muscle transplantation experiments (p. 516).

It is evident from these results that true limb function cannot be obtained from eye muscle centers, and Nicholas' results must therefore be interpreted in a different light. We do not contend that the analysis of cases of this sort has been carried to the point where no further clarification is needed, but we hope to have shown that they are not necessarily inconsistent with the resonance principle.

Returning to the normal emission system for a limb, confined, as we have seen, to the normal limb level of the cord, the question arises, as to whether it is equipotential throughout or rather contains discrete localized centers producing the specific discharges for the various muscles, one center for each muscle. We have found that the modulated nerve of any limb muscle may obtain its proper call from any of the three limb segments. Therefore, if there are discrete muscle centers, they must consist of longitudinal cell columns extending throughout the whole length of the three limb segments. In mammals the existence in the spinal cord of a central representation of individual muscles or groups of such in the form of longitudinal nuclei has been claimed (Sano, 1898; Marinesco, 1901, van Gehuchten & Nelis, 1899), but has more often been contested (Lapinski, 1904; Bikeles, 1905; Déjérine, 1909). In Amphibia nothing of the sort has ever become known, and in view of the primitive organization and the comparatively low number of cells in the cross-section of the amphibian spinal cord such an assumption seems very unlikely.

In two animals an attempt was made to approach the problem experimentally (P.W. 1936 b). Each animal had a normal forelimb, innervated from the 3rd and 4th nerves, and a supernumerary limb innervated from the 5th nerve. After the transplants had become perfectly functional and their homologous response with the normal limbs had been ascertained, the animals were decerebrated, whereupon the spinal reflexes continued to show homologous response. Then, penetrating from the medulla, the spinal cord was destroyed in successive steps. When the destruction was carried just beyond the 4th segment, the intact limb was paralyzed while the transplant, connected with the preserved 5th segment of the cord, was still functional. The reflex function exhibited by these transplants was amazingly complete and co-ordinated, indicating that the surviving fragment of the spinal district had been in possession of the full co-ordinating mechanism for a limb. This suggests equi-

potentiality of the central action system within the spinal limb district, although one would hesitate to attribute much significance to these first exploratory cases.

The claim that dynamic rather than topographic peculiarities determine the patterns of central activities has often been advanced; for instance, by J. Loeb (1899), Bethe (1925), Lashley (1929), Köhler (1930), to mention only a few. Our investigations, while from the beginning strongly supporting this view in a general sense (1928 *a*, pp. 80 and 144) have not yet contributed to elucidate the nature of the central action system, except very vaguely, first, by limiting the possibilities to such types of dynamics as are capable of assuming the various specific forms or modes postulated by the resonance principle, and, second, by refuting the idea that integration may chiefly consist of switch work along definite chains of neurones. For the rest, the possibilities are so numerous that it does not seem appropriate, at present, to venture any hypothesis. R. Lillie (1936¹) has recently illustrated by means of a model how resonance phenomena might appear in a nervous system regardless of topographical relations, on an electrical basis. But we deliberately refrain from further speculation in this matter as long as so much pertinent information is lacking.

For reasons which cannot be detailed here, it does not seem very likely that the activity of the central action system should consist mainly of controlled conduction (propagated disturbance) in nervous elements as which it has emerged from Sherrington's work (1906). To judge from the spiked action potentials indicating propagated disturbance, there can be no doubt but that a great deal of mere conduction of the peripheral type is met with in the centers. But we must remain awake to the possibility that the elements manifesting this activity may consist of modulated neurones, those second, third and higher neurones which possibly through a progressive modulation from the periphery have functionally become an extension of the periphery into the centers (see above, p. 515). From this standpoint, the action potentials led off from a central auditory tract (Saul & Davis, 1932) or optical tract (Kornmüller, 1932; Fischer, 1934), would be indicative of peripheral activity carried into the centers rather than of true central activity, which latter may conceivably not even be associated with action potentials of the spike type at all. Spike potentials would be found before an afferent excitation has been translated into an appropriately specific central effect, and after a change in the central action system has activated an appropriate motor set of fibers; but between the two there may be a gap in conduction. If fields of central neurones are synchronized in their action by some agent (Adrian & Matthews, 1934), this agent is certainly not itself conducted by propagated disturbance. When conduction in a neurone is interfered with during its mere passage without synapse through spinal gray matter, as demonstrated by Barron & Matthews (1935), this interference, indicative of the activity of the central action system, is not in itself of the nature of conducted disturbance. Thus, the conception of a dual mechanism involved in the nervous system—central activity versus conduction in fibers—receives constantly increasing support; to this the

¹ *Biological Reviews*.

resonance principle adds the information as to how the two sides are linked and in communication with each other.

Since the resonance principle characterizes solely the relationship between the central action system as such and the periphery, it bears no information concerning the intrinsic mechanism of the central activity itself, and the fundamental problem of central integration has received no immediate illumination from our experiments, except in one respect: namely, the relation between integrative action and plasticity. In the *Amphibia*, a given pattern of spinal emissions,—*e.g.* the one underlying the act of walking—although providing for a certain degree of freedom in its performance such as is necessary to meet the hazards of a normal environment (moving on uneven ground, dodging obstacles, etc.) is yet not plastic in the sense that it could be remodeled altogether in adaptation to exigencies resulting from an experimental alteration of the effector apparatus. Our demonstration that anatomically inverted legs function permanently in the wrong and reverse sense (P.W. 1935 *d*) has proved convincingly that in this case the pattern of integrated emissions as such remains unmodified, still correct from the standpoint of the original normal conditions which have ceased to exist, but hopelessly inadequate under the new abnormal conditions. This contains clear proof of rigidity rather than plasticity.

Manigk (1934), on the other hand, has reported an experiment which, in his interpretation, emphasized an almost unlimited adaptability of the locomotor co-ordination in *Amphibia* to unforeseen experimental situations. A frog whose hind legs were sutured together lengthwise down to the ankles, began to walk with its feet alternating, each foot moving with the contralateral arm. Surprisingly enough, this type of progression was found to persist even after the Achilles tendons of the two fused legs had been crossed, which seemed to indicate an amazing capacity of the central nervous system to find a suitable solution for the most difficult peripheral problems confronting it. But Taylor (1935), repeating and varying Manigk's experiments, came to the conclusion that the supposed adjustment of the scheme of co-ordination had merely been simulated by the mechanical effects of traction transmitted by the Achilles tendons, and that in reality the motor discharge pattern is the same, tendons crossed or uncrossed. There is, therefore, no evidence left in favor of the assumption that the disarrangement of the distribution of the muscles may entail a readjustment of the patterns of spinal co-ordination in *Amphibia*.

In higher vertebrates and in man, on the other hand, it is well known that after shifting the effective insertion of a muscle on the skeleton, adjustments in the patterns of co-ordination ensue which eventually succeed more or less in making the dislocated muscle co-operate in the general functional pattern of the body as a whole. The literature on this subject is too extensive to be reviewed here (compare Bethe and Fischer, 1931; Bethe, 1931). With our experiences on *Amphibia* in mind, one wonders whether these readjustments in higher vertebrates may not be purely a matter of cerebral activities capable of superseding spinal patterns after they have become inadequate; if this is the case, decerebration should at once bring out again the original wrong pattern. I am not aware that this test has ever been applied.

It results from the discussion that, if we grant to the central action system equipotentiality and free dynamic play including a certain adaptive behavior in the sense of Bethe's "plasticity", we must clearly distinguish between the alternative utilization of normal motor patterns in subservience to a general functional plan of the whole, on the one hand, and the remodeling of those normal motor patterns in order to remedy inadequacies arising from a disarrangement of the muscles, on the other. While the former has been amply demonstrated by Bethe (1930) and his collaborators (Bethe and Woitas, 1930; v. Holst, 1934), no evidence for the latter seems thus far to have been forthcoming except in animals possessing a brain cortex.

. As a substratum for the central action system we may most logically claim the neuropil of the spinal gray matter. Many of the characteristics ascribed by Herrick (1930, 1934) to the various neuropils of the amphibian brain cover almost precisely the requirements which our central action system must be expected to fulfill according to our definition: a diffuse matrix communicating with the localized (specialized) fiber tracts, receiving excitations from the central ends of the sensory neurones and discharging again into the central ends of the motor neurones, intercalated between both as an interruption of chains that are conventionally thought of in terms of uninterrupted reflex arcs. Although special studies on the spinal cord of amphibia are wanting, there is good reason to believe that it contains a neuropil essentially similar in character to the ones found in the brain. The extent to which central dendrites and axones may belong to either the specified peripheral system or to the neuropil is an open question.

Inasmuch as the correspondence between centers and periphery is based on a dual principle, namely, specific emission by the central action system, on the one hand, and specific reception by the periphery on the other, every case in which muscular contractions are found lacking in specificity and co-ordination must be scrutinized in a twofold light. For, general and unspecific responses can be caused not only by undifferentiated emission from the central action system, which is the commonly accepted interpretation, but by the absence of selective power on the peripheral side as well. Our experiments with deviated nerves reported above on p. 517 illustrate how unspecific responses may be obtained in the presence of normal and specific emissions, owing to incomplete peripheral modulation. This point must be stressed here in connection with the discussion of the central action system because there is a tendency to ascribe similar effects to the ill-known central action system exclusively. For instance, all the various results of experiments and surgical studies on muscle translocation, tendon crossing, nerve crossing and nerve deviation have been complacently lumped together under the common heading of "re-education". We realize, however, that whereas an artificial change in the anatomical insertions of *muscles* would require a real reorganization of the central emission patterns in order to make the muscles again serviceable to the body, the apparent readjustment noticed after the interchange of *nerves* is nothing but the expression of the peripheral remodulation of the nerve, without there being any change necessarily involved in the central action system. The point has been stressed on previous occasions (P.W. 1928 a, p. 138). A clear distinction between central adjustments

and peripheral modulation would greatly facilitate, and therefore should henceforth guide, the evaluation of experimental and clinical facts.

This distinction is of equal importance when we come to consider the embryonic development of nervous functions. To Coghill goes the credit for having called particular attention to this problem (Coghill, 1929). The study of incipient neuromuscular function in Amphibia revealed a gradual transition from a stage of generalized mass function of the entire innervated periphery to a final stage of localized and differentiated activity of individual organs. This transition was ascribed to a progressive individuation of local central patterns within the originally generalized pattern of the whole; in other words, it was considered, as if by necessity, as a purely central affair (Coghill, 1933). From our standpoint, however, it seems conceivable that at least part of the generalized and sweeping character of the early functions may be due to a certain lag of modulation of the peripheral nerves behind differentiation of the central action system. Since modulation is the outcome of the muscular differential, it cannot be expected to occur before the muscles themselves have reached a sufficiently advanced stage in their differentiation; prior to that critical stage only generalized responses could appear. One may anticipate considerable variability in regard to this critical stage among different groups of animals, and possibly this variation may partly account for the fact that the results obtained on the Amphibia (Coghill) and the rat (Angulo, 1932) do not check with the observations on the cat (Windle, 1934) and the chick (Orr & Windle, 1934).

V. SUMMARY

1. In 1922 it was discovered that a supernumerary transplanted limb in amphibia invariably duplicates the movements of the normal limb in whose nerve plexus it shares. This phenomenon, called "homologous response", has been demonstrated to be an expression of the more fundamental fact that, in every reflex and spontaneous action, multiple muscles of the same name (synonymous muscles), innervated from the same functional district and the same side of the spinal cord, contract simultaneously and with approximately equal intensities, regardless of their anatomical position, the details of origin and distribution of their nerve supply, and the functional effects of their contractions with regard to the body as a whole. Homologous response of synonymous muscles, observed under such a variety of different conditions as enumerated in Section II (1), has led to the realization that the linkage between the nervous centers and the non-nervous periphery is based upon mutual relations discriminative for each peripheral organ and much more specific than had previously been suspected. Further experimental analysis of this relationship has led to the following conclusions.

2. The specific relations between muscles and centers are not secondarily established by adjustments of the "conditioning" or "learning" type; for, the principal features of the latter, modifiability and adaptive functional value, are absent in the phenomenon of homologous response. In fact, the phenomenon is not even

dependent upon the presence of sensory control at all, since it is likewise obtained from limbs with purely motor innervation (III (1)).

3. Nor is the specific relation between muscles and centers the result of morphological selectivity in the formation of peripheral nerve connections. Abundant evidence is on hand showing not only that in general no affinity whatever exists between a given nerve fiber and a particular muscle, but more specifically, that such an assumption can be decisively ruled out for the experimental cases exhibiting homologous response. Many new facts have been added in substantiation of this point and are discussed in Section III (2).

4. Other explanations being excluded, the specific relation between centers and muscles must be regarded as due to a primary physiological relationship, resembling in principle the specific linkage found in resonance-like mechanisms. Every muscle is constitutionally (presumably biochemically) different from every other non-homologous muscle, and to deal with this diversity the centers are endowed with a capacity to produce a corresponding variety of forms or modes of motor impulses, each one exclusively appropriate to a single muscle. Thus, the activation of a muscle becomes a matter of dual activities: release of discriminative emissions from the centers, and the selective reception of these by the periphery.

5. Experiments involving the transplantation of single supernumerary muscles in adult toads (IV (1) a) have indicated that the peripheral muscle-specific selection does not actually occur in the muscle fiber itself but in some part of the peripheral nervous system acquiring its muscle-specific selectivity through a specifying ("modulating") effect extending from the muscle. It is assumed that motor neurones subjected to these peripheral influences are gradually rendered specific to such an extent that they will no longer admit from the centers motor impulses other than those appropriate for the particular muscle at their peripheral ends. A study of the reappearance of motility in denervated and re-innervated limbs in the toad has revealed that the specification of a nerve by its muscle is a slow process. Specification is, at least up to a certain age, reversible, the connection of a nerve with a new muscle resulting in corresponding respecification. Thus, the specific diversity of the muscles is projected into the motor nerves, and E. Hering's postulate of the diversity ("Ungleichartigkeit") of nerves becomes, after all, a fact.

6. Concerning the spinal centers, the conclusion has been reached that their functioning cannot be satisfactorily envisaged in terms of switch-board or other geometrical schemes (IV (2)). We have been forced to concede to the limb center, for instance, the property to produce, release, and circularize within certain intracentral limits, a variety of specific effects matching the existing variety of individual limb muscles. To judge from experimental evidence, the release of impulses specific for limb muscles is confined to the limb level of the spinal cord.

7. It has not yet been possible to offer any suggestion as to the presumable nature of the described selective affinity between central impulse and peripheral nerve fiber. But reasons have been advanced which, it would seem, allow us to reject the idea that specific frequencies or specific chronaxie relations (isochronism) are involved.

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ADDENDUM (5 June 1936)

While this article was in press, I have learned of the interesting experimental work of P. Anokhin and collaborators published in a recent book: *Reports on the problem of centre and periphery in the physiology of nervous activity*. Gorky State Publishing House, 1935. I regret that this work has come to my attention too late to be discussed on the present occasion, for it has a most intimate bearing on the problems dealt with in this article. Largely concerned with the functional results of nerve crossings in mammals, the experiments reported furnish valuable illustrations of the phenomenon described above (p. 514) as despecification and remodulation of nerves disconnected from their old and reconnected with a new periphery. Viewed from this angle the general results from Anokhin's laboratory are not only not contradictory to the conception outlined in this article, but supply pertinent supplementary data in support of it.

